EDAR7

Environmental Dimension of Antimicrobial Resistance

Montréal • • • • • May 26-31 2024

Presented by

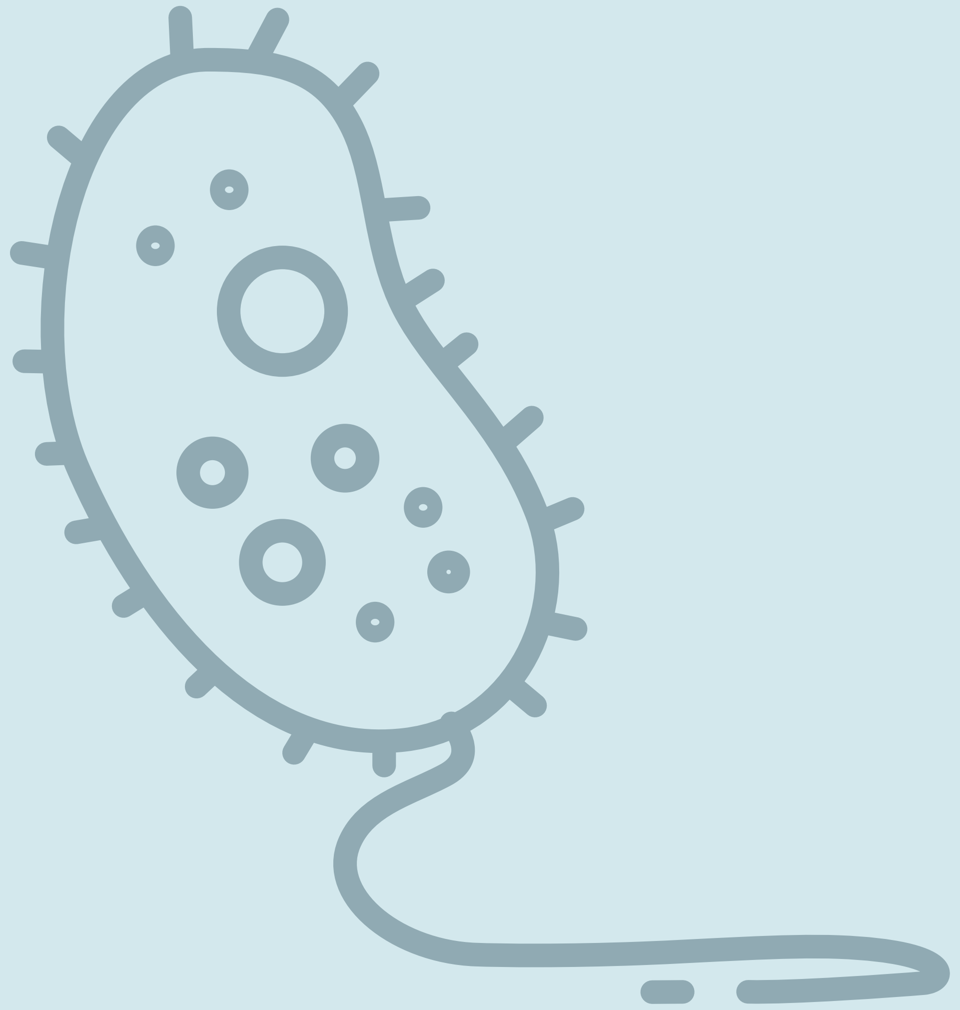
McGill

AMR
MCGILL
ANTIMICROBIAL
RESISTANCE
CENTRE

RAM
CENTRE
DE RÉSISTANCE
ANTIMICROBIENNE
DE MCGILL

Full Program

Contents



Word of Welcome

Dear friends and colleagues,

We are delighted to welcome you to Montréal for the 7th Environmental Dimension of Antimicrobial Resistance conference (EDAR7). Antimicrobial resistance (AMR) is a seminal global challenge that is threatening public health, food security, and economic prosperity. The importance of the environmental dimension of AMR is now recognized by national and international mandated authorities in the context of One Health.

The Local Organising Committee and the Scientific Program Committee were very active to bring you a program that will generate discussions and foster collaborative actions among the participants. Effective policies and actions to combat AMR across the One Health continuum must be evidence-based. Foundational information is required on the ecology and biology of AMR, factors driving the development and transmission of AMR, the importance of current practices in the different use sectors, and validation of the most effective mitigation and stewardship practices.

The veterans of the conference will recognize a number of new initiatives that were introduced to facilitate the participation of all. For example, we introduced parallel sessions for part of the day and flash poster presentations to provide broader opportunities for presenters while keeping up with the growth of the number of participants. An exciting workshop program was also developed by you (the participants) that will address important current scientific and policy questions. In step with the EDAR tradition, these grassroot initiatives will hopefully catalyse the interdisciplinary actions of our community and offer new occasions for incomers to rejuvenate our efforts to propose sustainable solutions to the AMR crisis for protecting the environment and human health.

Montréal is a dynamic bilingual cosmopolitan city, is the second largest city in Canada and the economic engine of the province of Québec. We hope that you will enjoy all that the city has to offer, and hopefully explore farther afield before or after the conference.

Many thanks to our valued sponsors, volunteers and our professional conference organizer JPdL, without whom the event would not have been possible.

We sincerely look forward to sharing and working with you all in Montréal!

Best wishes,

Prof. Dominic Frigon
Chair of EDAR7
Associate Professor
Dept of Civil Engineering
McGill University

Dr. Ed Topp
Co-chair EDAR7
Senior Research Chair, French priority program onAMR
UMR Agroécologie, INRAE
Université de Bourgogne Franche-Comté

Word from AMR Center

Dear colleagues and EDAR7 conference guests,

On behalf of the McGill Antimicrobial Resistance (AMR) Center, it is my great pleasure to welcome you to the 7th Environmental Dimensions of Antimicrobial Resistance (EDAR7) conference in Montreal. The McGill AMR Centre brings together a diverse scientific community with expertise across the biomedical, engineering, animal, natural and social sciences. Our mission is to harness the expertise and creativity of our researchers to address AMR challenges and priorities through interdisciplinary and collaborative research and education (https://www.mcgill.ca/amrcentre/). By hosting EDAR7, we are proud to contribute to the ongoing global efforts to curb the AMR crisis.

The Local Organising Committee and the Scientific Program Committee have planned a rich and diverse program that promises to be stimulating and productive. The event brings together leading scientists and trainees, policymakers and stakeholders from across the world to share insights, present leading research, and discuss strategies to address the complex issues of AMR in the environment at the intersection of human and animal health. Kudos to Profs. Frigon and Topp, members of the Local Organising Committee and the Scientific Program Committee, administrative support team and volunteers for your tremendous efforts and exciting program.

I hope that you will leave this conference with a broadened perspective, fresh ideas, new collaborations, and actionable plans for your continued and dedicated work to address the AMR crisis. Thank you for joining us in Montréal. I wish you a stimulating conference and enjoyable stay in our city.

Dao Nguyen
Director, McGill AMR Centre
Department of Medicine
McGill University

Steering Committee Members

Dominic Frigon
McGill University

Edward Topp
INRAE, Université de Bourgogne

Dao Nguyen
McGill University

Paul Thomassin
McGill University

Xin Zhao
McGill University

Jennifer Ronholm
McGill University

Sebastien Faucher
McGill University

Emilie Bédard
Polytechnique Montréal

Dominic Poulin-Laprade
Agriculture and Agri-Food Canada

Sukriye Aydin
McGill University

Sybille Brault
Tourisme Montréal

Angela Casey
McGill AMR Centre

Helena Toledo
Université de Montréal

Scientific Committee Members

Amrita Bharat
Public Health Agency of Canada

Kristian Brandt
University of Copenhagen

Jordyn Broadbent
Environment and Climate Change Canada

Juliana Calábria de Araújo
Universidade Federal de Minas Gerais

Carolee Carson
Public Health Agency of Canada

Steve Djordjevic
Sydney Technical University

Erica Donner
University of South Australia

Sabiha Essack
University of KwaZulu-Natal

Dominic Frigon
McGill University

Jay Garland
U.S. Environmental Protection Agency

David Graham
Newcastle University

Joakim Larsson
University of Gothenburg

Ramanan Laxminarayan
One Health Trust

Jeff Lejeune
Food and Agriculture Organization of the United Nations

Ernesto Liebana
The European Food Safety Authority

Célia Manaia
Universidade Católica Portuguesa

Alison Mather
Quadram Institute & University of East Anglia

Rebecca Shapiro
University of Guelph

Kornelia Smalla
Julius Kühn Institute

Edward Topp
Institut national de recherche pour l’agriculture, l’alimentation et l’environnement (INRAE) & Université Bourgogne-Franche-Comté

Tong Zhang
University of Hong Kong

Yong-Guan Zhu
Chinese Academy of Sciences

Heike Schmitt
National Institute for Public Health and the Environment (RIVM), Delft University of Technology, Wetsus,

Joanne Elmoznino
Pfizer

THANK YOU TO OUR PARTNERS

Host

McGill | AMR McGILL ANTIMICROBIAL RESISTANCE CENTRE RAM CENTRE DE RÉSISTANCE ANTIMICROBIENNE DE McGILL

Major Contributor

Public Health Agency of Canada Agence de la santé publique du Canada

Platinum Sponsor

resistomap

POWER CORPORATION OF CANADA

Silver Sponsor

Montréal business events

Bronze Sponsor

GenomeQuébec

GenomeAlberta

Friends

CentrEau
Centre québécois de recherche sur l'eau
Quebec Water Research Centre

Centre de Recherche en Infectiologie Porcine et Avicole
Swine and Poultry Infectious Diseases Research Center
CRIPA
Université de Montréal

McGill | M[i]4 Interdisciplinary Initiative in Infection and Immunity

Fondation du Centre universitaire de santé McGill
McGill University Health Centre Foundation

CSM Canadian Society of Microbiologists
SCM Société canadienne des microbiologistes

BIOaerosols

CAWQ
ACQE

PROGRAM AT A GLANCE

EDAR7
Environmental Dimension of Antimicrobial Resistance

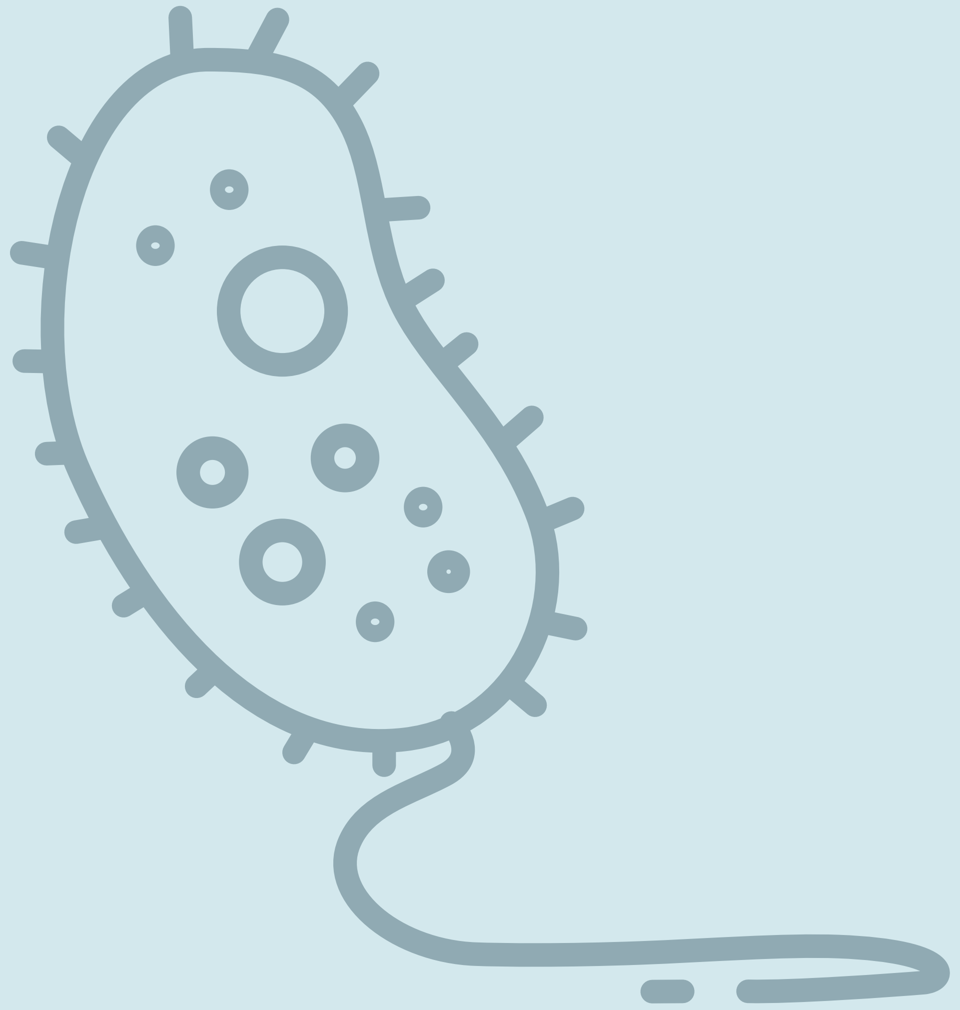
			SUNDAY MAY 26	MONDAY MAY 27	TUESDAY MAY 28	WEDNESDAY MAY 29	THURSDAY MAY 30	FRIDAY MAY 31
8:30 - 10:15				Session 2: Microbial Diversity Carrying Acquired Resistance *Invited Speaker: Joakim Larsson* Lectures Flash Orals	Session 7: AMR and the world's polycrisis **KEYNOTE: Prof. Sabiha Essack** Lectures Flash Orals	Session 14: Integrated surveillance along the One Health continuum *Invited Speaker: David Graham* Lectures Session 15: Technical Exhibitor Session Resistomap,Qiagen, Illumina	Session 16: One Health AMR biosecurity and mitigation approaches *Invited Speaker: Lance Price* Lectures Flash Orals	Session 21 *Invited Speaker: Anthony So* Workshop reports and discussion
10:15 - 11:00				Break	Break	Break	Break	Break
11:00 - 12:30	REGISTRATION (11:00)			Session 3: Environmental Management & AMR *Invited Speaker: Caroline Duchaine* Lectures Flash Orals Session 4: Culturomics vs. Metagenomics for Surveillance *Invited Speaker: Amy Pruden* Lectures Flash Orals	Session 8: Evolution of AMR *Invited Speaker: Jianhua Guo* Lectures Flash Orals Session 9: Modeling AMR Threats *Invited Speaker TBC* Lectures Flash Orals	One Health Panel Discussion *Genome Quebec - Public Health Agency of Canada* Pre-Worshop Communication *WHO - Quadripartite Secretariat*	Session 17: Environmental Pollution impact of Development and Fate of AMR *Invited Speaker: Steven Djordjevic* Lectures Flash Orals Session 18: Wastewaterbased surveillance and link to human populations *Invited Speaker: Heike Schmitt* Lectures Flash Orals	Workshop reports and discussion **CLOSING CEREMONY**
12:30 - 13:45				Lunch	Lunch	Lunch	Lunch	
13:45 - 15:30		EXHIBITION HALL OPENS (15:00)		Session 5: Fate of AMR During Wastewater Treatment *Invited Speaker: Juliana Calábria de Araújo* Lectures Flash Orals Session 6: Impact of bacteriophage and predators on AMR *Invited Speaker: Pedro Alvarez* Lectures Flash Orals	Session 10: AMR Threats in Agriculture *Invited Speaker: Yong-Guan Zhu* Lectures Flash Orals Session 11: Bioinformatics of ARGs *Invited Speaker: Tong Zhang* Lectures Flash Orals	Workshops	Session 19: Wildlife and other environmental reservoirs of AMR *Invited Speaker: Monika Colejska* Lectures Flash Orals Session 20: Predicted NoEffect Concentrations of Antimicrobials *Invited Speaker: William Gaze* Lectures Flash Orals	
15:30 - 16:00				Break	Break	Break	Break	
16:00 - 17:00			Session 1: OPENING PLENARY **KEYNOTE: Prof. Gerry Wright** Welcome and Dignitaries	Poster Session A	Session 12: Biosynthesis of Antimicrobials *Invited Speaker: Eszter Farkas* Lectures Session 13: Resistance to antifungals and other antimicrobials *Invited Speaker: Johanna Rhodes* Lectures	Workshops	Poster Session B	
17:00 - 18:00								
Evening			EDAR7 Delegate Welcome Reception		Gala Dinner	Event for Public Outreach (EDAR attendees invited)		

Sunday, May 26 - Room Montréal (Mtl) 3-4

Session 1 (16:00-18:00) : Opening Plenary

OPENING PLENARY - Keynote Speaker

The Natural History of Antibiotic Resistance
Prof. Gerry Wright



Monday, May 27 - Room Montréal (Mtl) 3-4

Session 2 (8:30-10:15) : Microbial Diversity Carrying Aquired Resistance

Where do antibiotic resistance genes become mobile?
Invited Speaker: D. G. Joakim Larsson

Presentations

Prevalence and transmission of antibiotic resistance in wastewater treatment systems
Dou Wang - Zhang, T.

Genomic disparities in extended-spectrum β-lactamase-selected versus non-selected Escherichia coli from Australian wastewater
Veronica Jarocki - Bogema, D.; Li, D.; Yam, J.; Jenkins, C.; Hai, F.; Djordjevic, S.

Identification of metabolic and temporal niches and their association to the persistence of antibiotic and biocide resistant bacteria in wastewater.
Orestis Kanaris - Kreft, J. U.; Schulz, A. C.; Nordholt, N.; Schreiber, F.

Clustering in Context: Antimicrobial Resistance and MGE Dynamics in ESKAPE Pathogens Circulating across Environmental and Used WWTP Waters in Romania
Marius Surleac - Czobor Barbu, I.; Paraschiv, S.; Gheorghe-Barbu, I.; Rotaru, L. I.; Otelea, D.; Chifiriuc, C.

Flash Poster Presentations

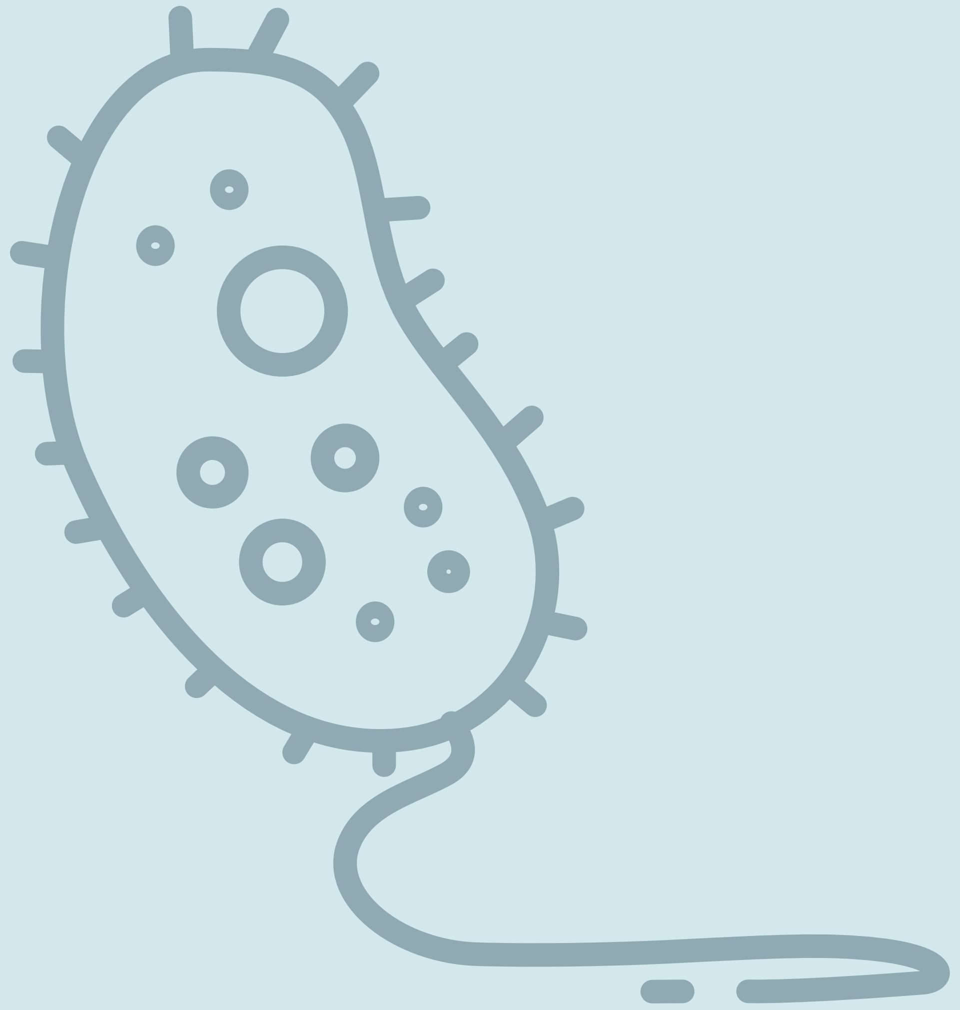
Spatial and temporal spread of antimicrobial resistance in aquatic environments
Máté Vass - Bengtsson-Palme, J.

Below the surface: The hidden consequences of eutrophication on river resistome, mobilome and virulome
Mira Cooper-Beknazarova - Opoku, S.; Donner, E.; Wyrsch, E.; Cummins, M.; Djordjevic, S.; Vasileiadis, S.; Awad, J.; Chow, C.; Saint, C.; Drigo, B.

Trimethoprim resistance in surface and wastewater is mediated by contrasting variants of the dfrB gene
David Kneis - Lemay-St-Denis, C.; Cellier-Goetghebeur, S.; Elena, A. X.; de la Cruz Barron, M.; Tskhay, F.; Heß, S.; Berendonk, T. U.; Pelletier, J. N.

Phenomic analyses as means to identify drivers of selection in hospital wastewater
Noel Waters - Larsson, J.; Coertze, M.; Ebmeyer, S.; Kristiansson, E.; Flach, C. F.; Fick, J.

Sequencing of ESBL E. coli from manure across four countries reveals high prevalence in all regions but distinct resistance patterns.
Nicole Ricker - Chalmers, G.; Glaeser, S.; Popa, M.; Scott, A.; Topp, E.; Sefeedpari, P.; de Roda Husman, A. M.; Schmitt, H.



Monday, May 27 - Room Montréal (Mtl) 4

Session 3 (11:00-12:30) : Environmental Management & AMR

Bioaerosols and Antibiotic Resistance Gene Transport: Understanding the Role, Impacts, and Fate
Invited Speaker: Caroline Duchaine

Presentations

Antimicrobial resistance dissemination potential of bio-based fertilizers according to field trials
Marko Virta - Markkanen, M.; Hultman, J.; Ylivainio, K.; Jansen, B.; Salo, T.; Delgado, A.; Recena, R.; Arkoun, M.; D'Oria, A.; Keskinen, R.;

Replacing untreated with treated wastewater for irrigation: Consequences for antibiotics, metals and nutrients in soils
Jan Siemens - Heyde, B.; Lüneberg, K.; Hahn, N.; Axtmann, K.; Sou-i, L.; Böckmann, M.; Braun, M.; Amelung, W.; Bierbaum, G.; Gallego, S.; Glaeser, S.; Grohmann, E.; Smalla, K.; Zarfl, C.; Siebe, C.

Trade-offs of increasing temperatures for the spread of antimicrobial resistance in river biofilms
Kenyum Bagra - Klümper, U.; Kneis, D.; Padfield, D.; Szekeres, E.; Teban-Man, A.; Coman, C.; Singh, G.; U. Berendonk, T.

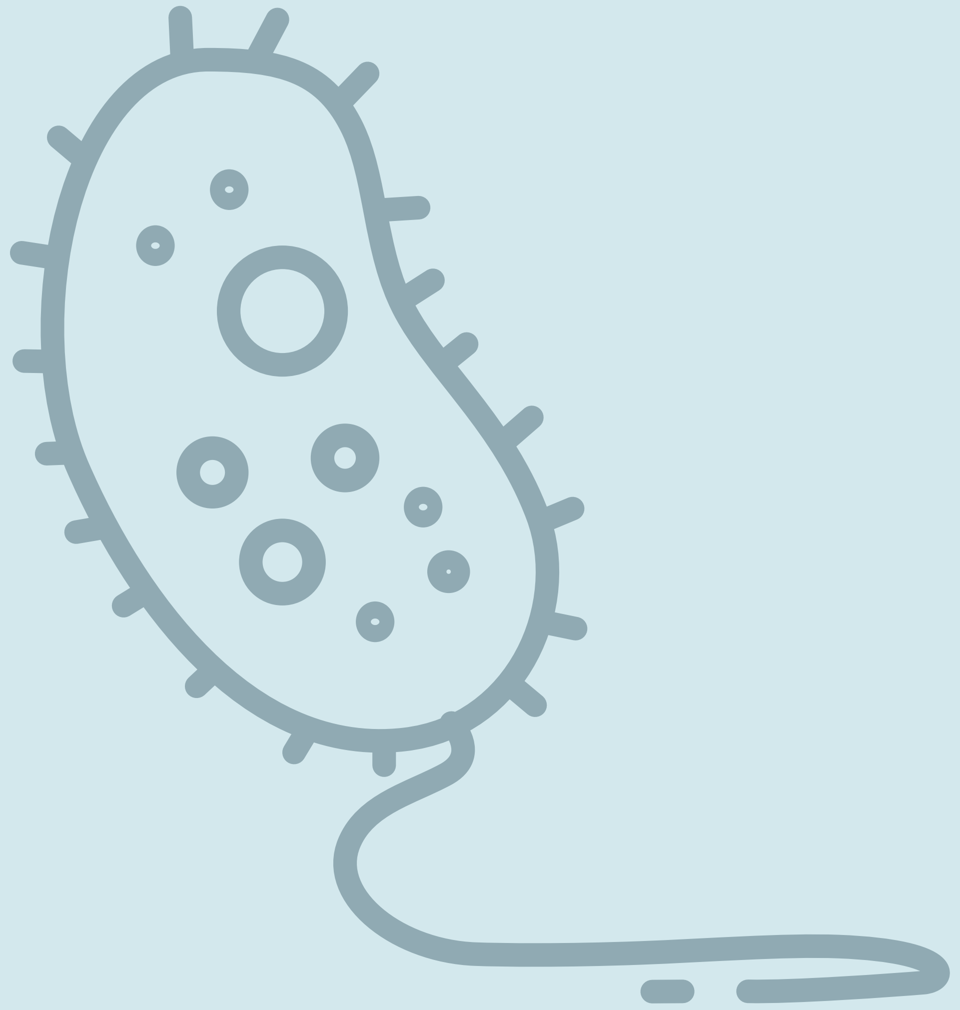
Flash Poster Presentations

Large-scale screening of antimicrobial resistance genes along 2300 km of the Danube River
Alexander Kirschner - Schachner-Groehs, I.; Koller, M.; Leopold, M.; Kolm, C.; Linke, R.; Jakwerth, S.; Kolarevic, S.; Kracun-Kolarevic, M.; Kandler, W.; Sulyok, M.; Vierheilig, J.; Toumi, M.; Farkas, R.; Toth, E.; Kittinger, C.; Zarfel, G.; Farnleitner, A.

Linking Antimicrobial Resistance Dissemination to Human Fecal Pollution: A Study on CrAssphage and ARGs in Hospital Wastewater and Environment
Prasert Makkaew - Siri, Y.; Sresung, M.; Paisantham, P.; Mongkolsuk, S.; Sirikanchana, K.; Honda, R.; Precha, N.

An analysis of wastewater antimicrobial resistance genes before and after the lifting of COVID-19-related travel restrictions
Jangwoo Lee - Gonzalez, C.; Au, E.; Xiang, K.; Du, K.; Waddell, B.; Acosta, N.; Bautista, M.; Bhatnagar, S.; Lee, B.; Pang, X.; Weyant, B.; Pitout, J.; Rennert-May, E.; Conly, J.; Hubert, C.; Parkins, M.

Key Elements within an Integrated Monitoring Framework of Antimicrobial Resistance for Asian Countries
Ryo Honda - Kumar, M.; Wang, R.; Mardalisa, M.; Sabar, M. A.; Chaminda, T.; Sirikanchana, K.; Makkeaw, P.; -, Sulfikar; Ju, F.; Jiang, G.; Li, B.; Chiemchaisri, C.; Gomi, R.; Amarasiri, M.; Venter, H.; Nishiyama, M.; Watanabe, T.; Ihara, M.; Kasuga, I.; Watanabe, K.; Suzuki, S.



Monday, May 27 - Room Montréal (Mtl) 3

Session 4 (11:00-12:30) : Culturomics vs. Metagenomics for Surveillance

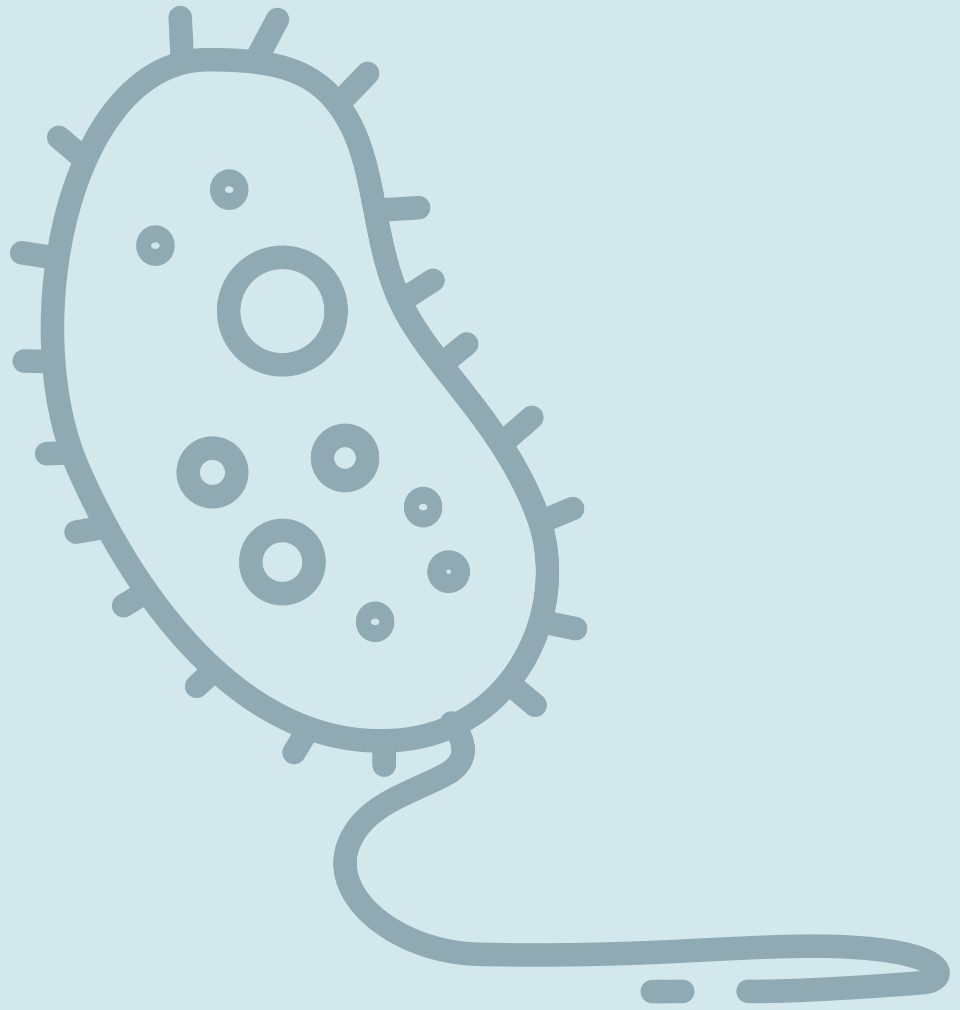
Antibiotic resistance genes (ARGs) as environmental contaminants: Where are we now?
Invited Speaker: Amy Pruden

Presentations

Cultivation strategies have a strong impact on studies of antimicrobial resistant bacteria
Stefanie P Glaeser - Kämpfer, P.; Pulami, D.; Balchandran, S.; Chifiriuc, C.; Schmitt, H.; Scott, A.; Topp, E.; Chalmers, G.; Ricker, N.

Bathing Water Based Epidemiology – five years AMR surveillance at Scottish bathing water sites (2018-2023)
Donald Morrison - Findlay, D.; O'Keefe, B.; Blaud, A.; Redshaw, J.

Genomic and metagenomic insights into antimicrobial resistance in human, poulty, and wastewater environements in urban and rural Bangladesh
Mohammad Islam



Flash Poster Presentations

Evaluation of the implementation of the strategic plan for surveillance and control of antimicrobial resistance 2018-2022 in Senegal.
Ibrahima Mamby KEITA - LEYE, M. M. M.; CISS, M.; DIOP, K. S.; WILANE, M. B.; SAMB, N.; DIALLO, A. A.; DIOUF, C.; NIANG, A.; NDIAYE, A. D.; SOW, A. I.; SECK, I.

Implementing a Surface Water Pilot within a National, One Health Focused Antimicrobial Resistance (AMR) Monitoring System in the United States (U.S.)
Jay Garland - Franklin, A.; McDermott, P.; Cook, K.

Phenotypic Metagenomics Reveals Wastewater-Associated Rare but Clinically Important Broad-Spectrum Beta-lactam Resistance Invading River Ecosystem
Guoqing Zhang- Buergmann, H.

Proximity-guided metagenomics to unravel physical association between plasmid-borne antimicrobial resistance genes and hosts in wastewater in Vietnam
My Hanh Vu Thi - Kurisu, F.; Suzuki, Y.; Hasebe, F.; Glidden, D.; Liachko, I.; Suzuki, M.; Kasuga, I.

Carbapenem-resistant Pseudomonas aeruginosa in hospital wastewater and the water chain in Indonesia and the Netherlands
Selvi Nafisa Shahab - van Veen, A.; Nguyen, G. Q. H.; Gauthier, J.; Kukavica-Ibrulj, I.; Potvin, M.; Kemper, M. A.; Rijfkogel, A.; Karuniawati, A.; Lévesque, R. C.; Severin, J. A.; Klaassen, C. H. W.; Schmitt, H.

Monday, May 27 - Room Montréal (Mtl) 4

Session 5 (13:45-15:30) : Fate of AMR During Waterwaste Treatment

Sewage Surveillance of Antibiotic Resistance in Brazil as revealed by long-term monitoring of wastewater treatment plants
Invited Speaker: Juliana Calábria de Araújo

Presentations

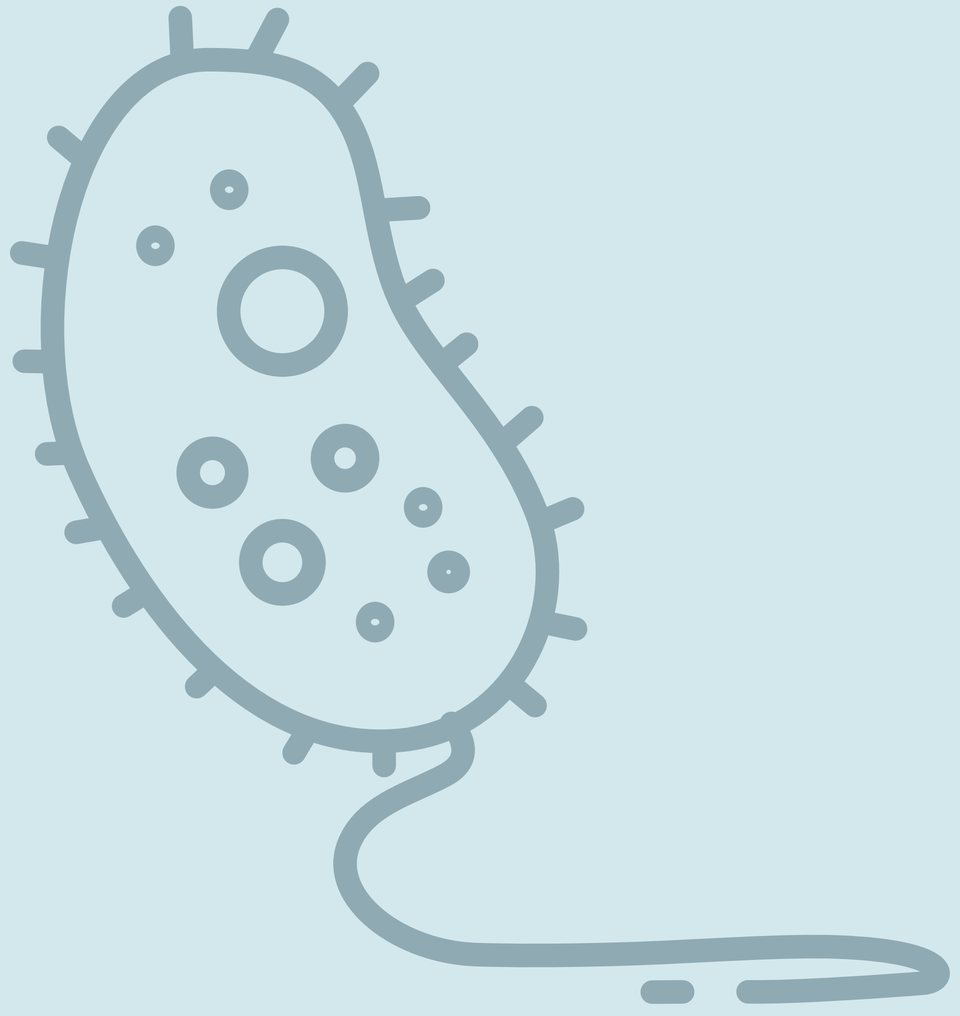
Diversity and dynamics of antimicrobial resistome in activated sludge across continents
Muhammad Adnan Sabar - Morinaga, Y.; Matsuura, N.; Watanabe, T.; Honda, R.

Complex Fate of Antimicrobial Resistance Genes in Activated Sludge Determined by Solids Retention Time
Claire Gibson - Frigon, D.

Exploring AMR in conventional and advanced wastewater treatment processes: A cross study along the oxygen gradient
Stella Christou - Calderón-Franco, D.; Nnorom, M.-A.; Liu, Y.; van Loosdrecht, M.; Guo, B.; Weissbrodt, D.; Ritchie, J.

Changes to antimicrobial resistance (AMR) following wastewater treatment processes: a UK-wide investigation
Holly Tipper - Newbold, L.; Read, D.; Johnson, A.; Kasprzyk-Hordern, B.; Daso, A. P.; Bowes, M.; Gweon, H. S.; Pereira, M. G.; Singer, A.

Flash Poster Presentations



Molecular epidemiology and mechanisms of carbapenem and colistin resistance in Klebsiella and Enterobacter from treated wastewater
Ana Puljko - Dekić Rozman, S.; Jelić, M.; Barišić, I.; Udiković-Kolić, N.

Compact decentralized advanced treatment methods to combat the release of AMR in wastewater effluents from AMR point-sources.
Carsten Ulrich Schwermer - Ingebretsen, T.; Olafssøn, H.; Xiaoyu, C.; Schwartz, T.

Impact of temperature, feedstock, and seasonality on antibiotic resistance genes in hyper-mesophilic anaerobic digestion of agricultural biomass
Bing Guo - Nnorom, M.-A.; Short, M.

Antibiotic resistome of final effluents from wastewater treatment plants across China
Li-Juan Li - Xiao, Y.; Zhao, B.-Y.; Chen, G.; Chen, W.; Yang, X.-R.; An, X.-L.; Su, J.-Q.; Zhu, Y.-G.

Antimicrobial Resistance in the Adyar River, India, and its Control by Electro-Oxidation
THARA M V - Vaidyanathan, R.; Nambi, I. M; Viswanathan, A.; Sanjeev, S.; Rathinavelu, S.; Jothi, A.; M, B.; J C, C.; Kreft, J.-U.; Pallarés Vega, R.; Graham, D.; Thatikonda, S.; Anurag, S.

Monday, May 27 - Room Montréal (Mtl) 3

Session 6 (13:45-15:30) : Impact of bacteriophage and preators on AMR

Proliferation and Phage-Based Control of Multidrug-Resistant "Superbugs" in Sewage Treatment Plants
Invited Speaker: Pedro Alvarez

Presentations

Phage Science, Therapeutics, and Research: The Public Health Agency of Canada's New Phage Therapy Program
Michael Parcey - Bharat, A.; Knox, N.; Mulvey, M.

Phagotrophic protists preserve antibiotic-resistant opportunistic human pathogens in vegetable phyllosphere
Chenshuo Lin - Li, L.-J.; Ren, K.; Zhou, S.-Y.-D.; Isabwe, A.; Yang, L.-Y.; Neilson, R.; Yang, X.-R.; Cytryn, E.; Zhu, Y.-G.

Protozoa predation: a major pathway of E.coli removal in aerobic granular sludge
Zhaolu Feng - Sutton, N.; Schmitt, H.; van Loosdrecht, M.

Paper-based microfluidic device for the detection of antimicrobial resistance genes in drinking water
Zhugen Yang - Pan, Y.

Flash Poster Presentations

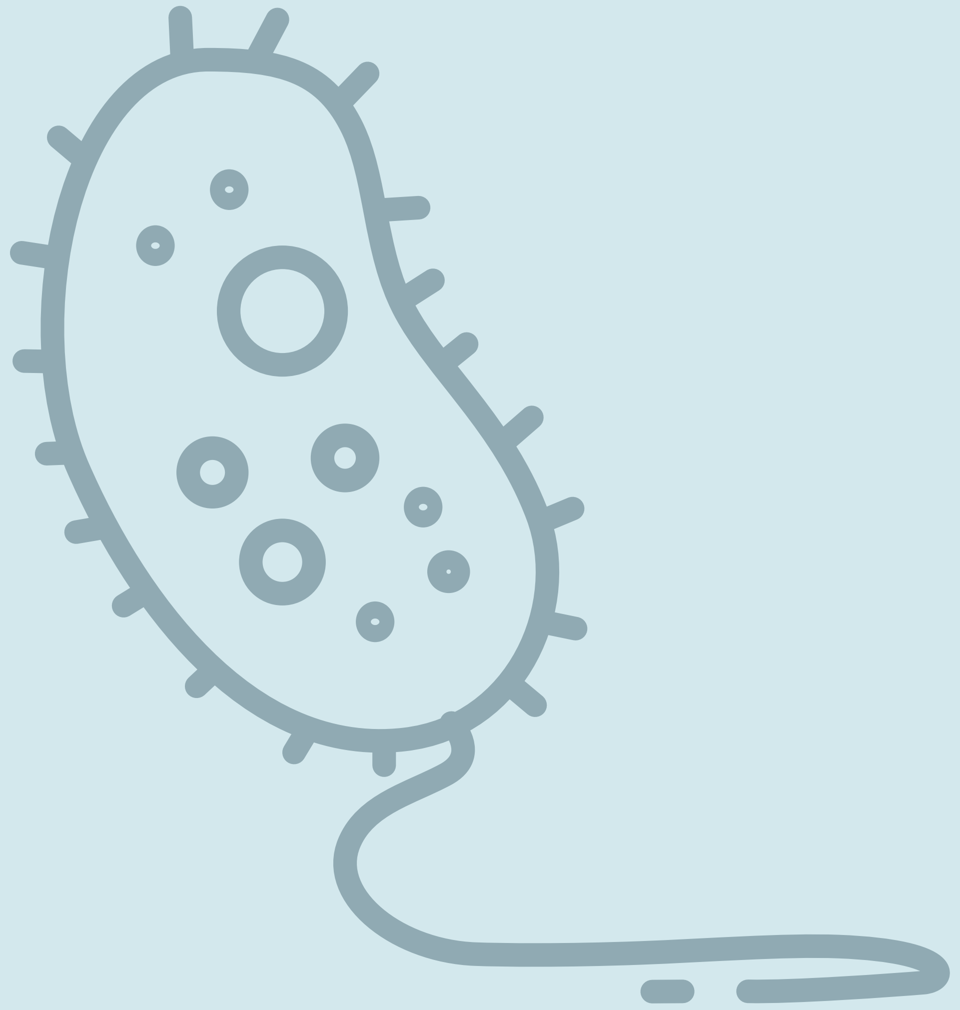
The alteration of microbial biodiversity in the receiving environment promotes its invasion by anthropogenic antibiotic resistance genes
Christophe Merlin - Catao, E.; Klemper, U.; Gionchetta, G.; Bellanger, X.; Dielacher, I.; Goryluk-Salmonowicz, A.; Szekeres, E.; Changey, F.; Walsh, F.; Woegerbauer, M.; Coman, C.; Popowska, M.; Vierheilig, J.; Bürgmann, H.; Berendonk, T. U.

A Systematic Review on Swine Pathogens in China: Trends, Prevalence, and Implications for Public Health and Biosecurity
Thanaporn Thongthum - Anderson, B.; Zhou, K.; Wang, J.

Profiling of Resistant Genes in Intermittent Water Distribution Systems and Household Water Storage
Gabriel Mesole - Chavarria, K.; Weston, S.; Kumpel, E.

Novel Cationic Antibacterial Polymers as Potential Antibiotic Replacements
Mary Chan-Park

Optimizing the Bovine Microbiome to Reduce Agricultural use of Antibiotics
Jennifer Ronholm - O'Brien, B.; Jung, D.; Park, S.; Cai, Z.; Nguyen, S.; Kurban, D.; Dufour, S.



Tuesday, May 28 - Room Montréal (Mtl) 3-4

Session 7 (8:30-10:21) : AMR and the world's polycrisis

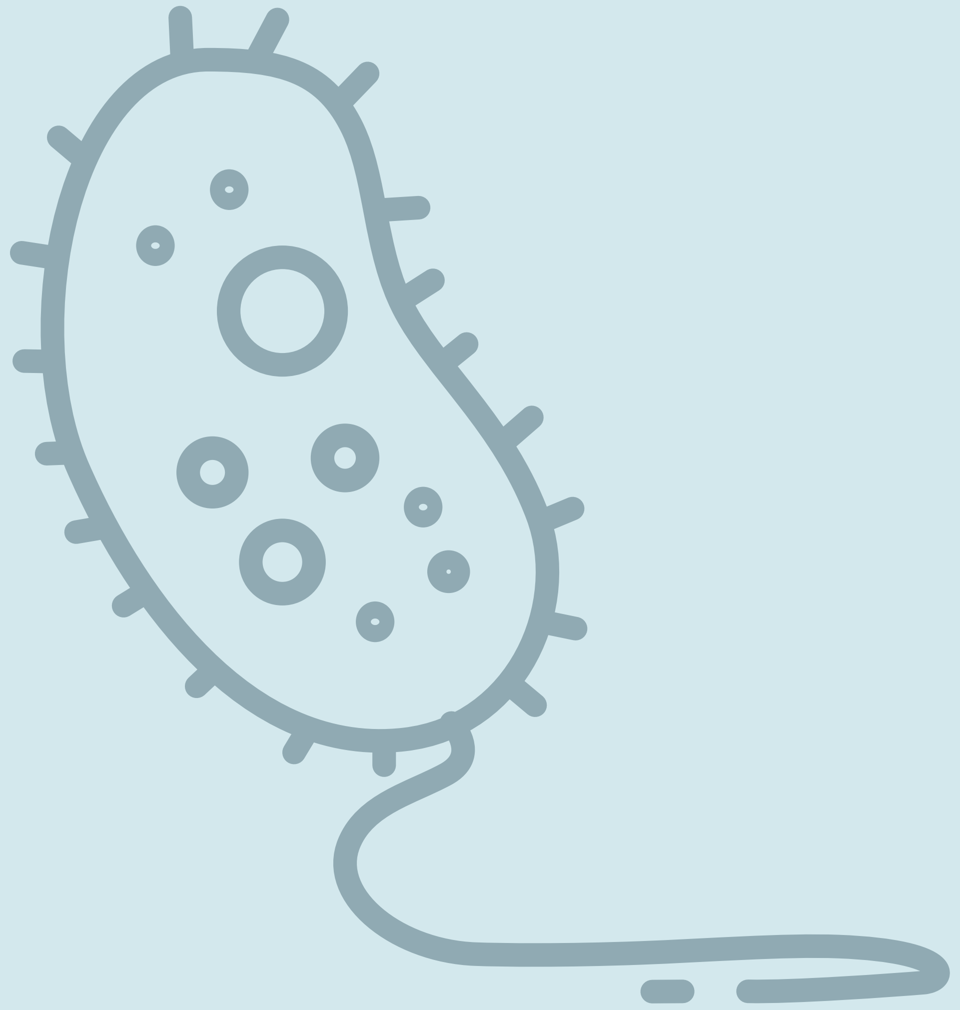
The Polycrisis and AMR: Global Policy Fails the Vulnerable
Keynote Speaker: Sabiha Essack

Presentations

Estimates of Human Exposure to Antimicrobial Resistance Foodborne Pathogens and the Resulting Burden of Illness
Yarina You - Reid-Smith, R.; Parmley, J.; Mukhopadhyay, K.; Thomassin, P.

Antimicrobial resistance National Action Plan goals translating into federal policies: A comparative analysis between the United States and Canada
Lucas Goodman - Schenk, T.

Combating the Spread of Antibiotic Resistance Negotiation Simulation: Using Serious Games to Simulate Policy Deliberation
Rebekah Riddle - Schenk, T.



Flash Poster Presentations

Antibiotic Resistance Genes in Wastewater and Environmental Water and Implications for Thailand National Action Plan on Antimicrobial Resistance
Kwanrawee Sirikanchana - Srathongneam, T.; Sresung, M.; Paisantham, P.; Raksakul, P.; Honda, R.; Singer, A.; Sukchawalit, R.; Satayavivad, J.; Mongkolsuk, S.

Opinions of stakeholders on the government, regulator and water industry response to the environmental challenges on the UK AMR National Action Plan
Isobel Stanton - Tipper, H.; Pacho, A.; Glover, R.; Singer, A.

Tuesday, May 28 - Room Montréal (Mtl) 4

Session 8 (11:00-12:30) : Evolution of AMR

Emergence, evolution and spread of antibiotic resistance associated with non-antibiotic drugs
Invited Speaker: Jianhua Guo

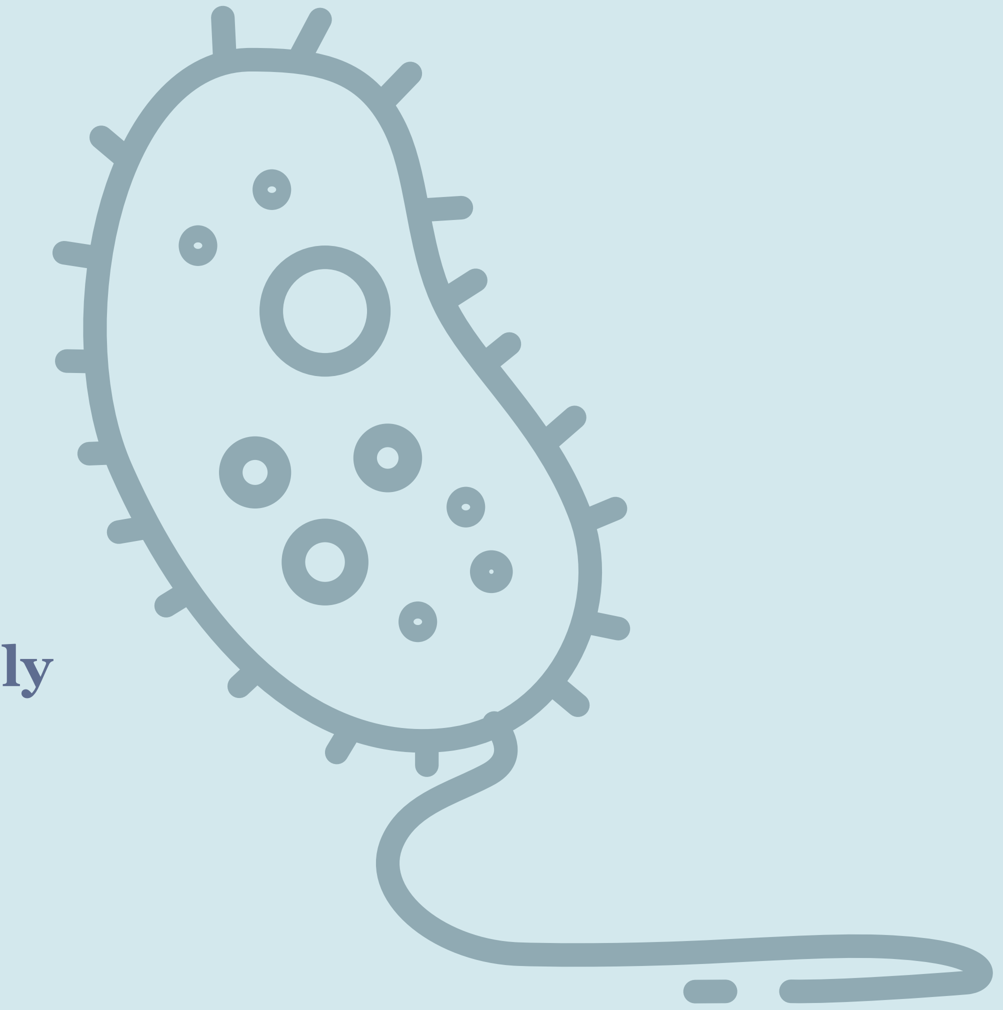
Presentations

Dynamics of class 1 integrons in wastewater treatment plants and hospital wastewater
Kaho Misawam - Yamamoto, T.; Amarasiri, M.; Maehana, S.; Furukawa, T.; Sano, D.; Sei, K.

Evolution dynamics of antimicrobial resistance under chlorination disinfection
Zhigang Yu - Guo, J.

The Role of Bacteriophages as Vectors of Antimicrobial Resistance in Wastewater Treatment Plants is Very Limited: A qPCR and Metagenomics-Based Study
Kadir Yanac - Yuan, Q.; Uyaguari, M.

Flash Poster Presentations



New epicPCR based system allows tracking of horizontal gene transfer of multiple resistance genes in a microbial community simultaneously
Veera Partanen - Virta, M.; Hiltunen, T.; Muurinen, J.; Karkman, A.; Dekić Rozman, S.

Distribution of antibiotic resistance between phages and bacteria in a river ecosystem receiving treated wastewater
Xavier Bellanger - Sagrillo, C.; Merlin, C.

The influence of predation by Tetrahymena borealis on conjugation in Aeromonas salmonicida subsp. salmonicida
Alicia Durocher - Paquet, V.; St-Laurent, R.; Charette, S.

Detection of plasmid-borne copper resistance genes in clinical isolate of Legionella pneumophila using comparative genomics
Maria Najeeb - Matthews, S.; Prevost, M.; Brodeur, J.; Cadieux, G.; Pilon, P.; Lalancette, C.; Faucher, S. P.

Soil colloids affect the selective effect of different antibiotics on bacterial growth
Katharina Axtmann - Brinkmann, S.; Siskowski, A.; Bierbaum, G.; Heyde, B.; Siemens, J.; Braun, M.

Tuesday, May 28 - Room Montréal (Mtl) 3

Session 9 (11:00-12:30) : Modeling AMR Threats

-

Invited Speaker: TBC

Presentations

Assessment of risks associated with antibiotic-resistant E. coli through consumption of produce irrigated with reclaimed wastewater
Hunter Quon - Hamilton, K.

Combining field studies, data and modelling to inform a Bayesian Network of risk to human health from environmental AMR
Jan-Ulrich Kreft - Troldborg, M.; Hough, R.; Uluseker, C.; Safi, W. H.; Modi, A.; Janivara Chandregowda, C.; Mohapatra, P. K.; Kuiry, S. N.; Larsen, J.; Sonkar, V.; Kashyap, A.; Chandrakalabai Jambu, S.; Srivastava, R.; Sasidharan, S. S.; Rengan, A. K.; Methale V. Paramba, T.; Rathinavelu, S.; Viswanathan, A.; Vaidyanathan, R.; Nambi, I. M.; Pallares Vega, R.; Ovis Sanchez, J. O.; Harwood, C.; Graham, D.; Thatikonda, S.

MetaCompare 2.0: Differential ranking of ecological and human health resistome risk
Monjura Afrin Rumi - Oh, M.; Davis, B. C.; Juvekar, A.; Brown, C. L.; Vikesland, P. J.; Pruden, A.; Zhang, L.

Flash Poster Presentations

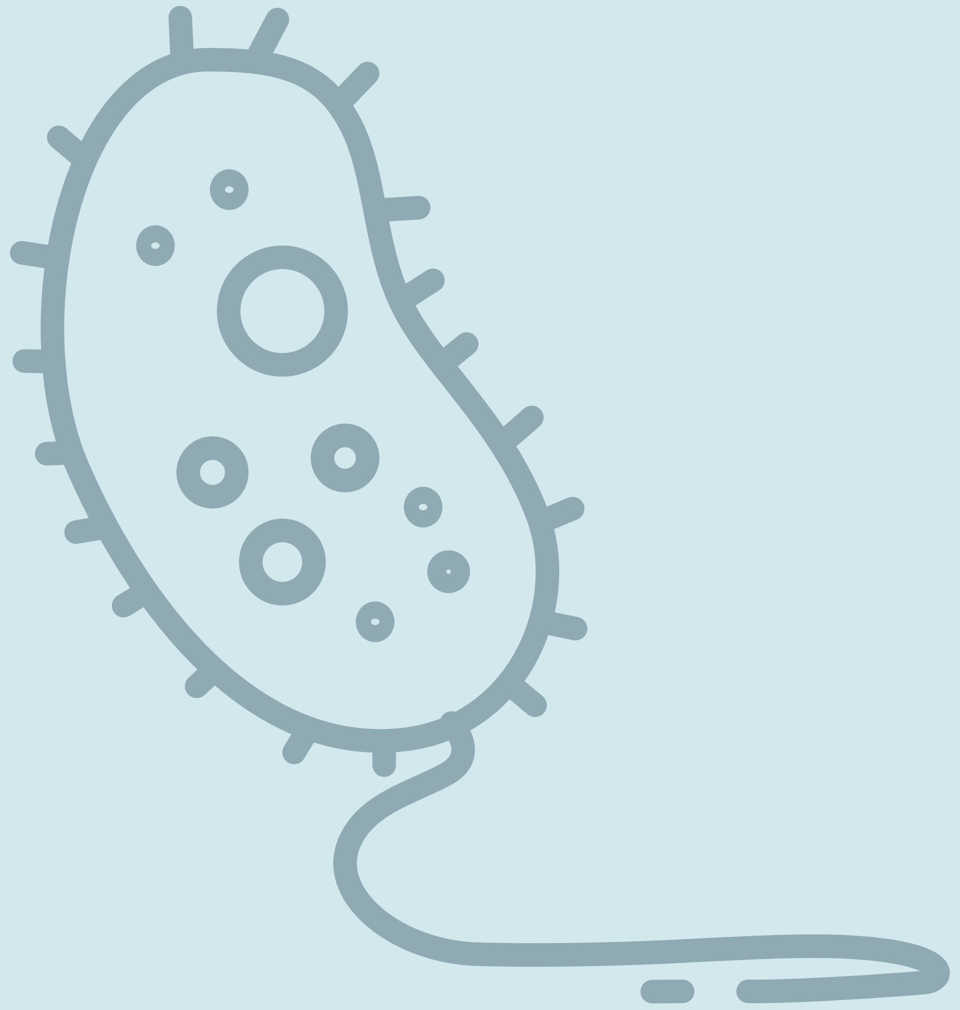
Hydrodynamic model-assisted tracking of antimicrobial resistance distribution in Lake Geneva
Sasikaladevi Rathinavelu - Beck, K.; Bouffard, D.; Irani Rahaghi, A.; Buergmann, H.

Elucidating the Transport and Fate of Antibiotic Resistance Genes from Fresh and Treated Cattle Manure in Freshwater Streams
Andrei Badilla-Aguilar - Ginn, O.; Vu, K.; Oceguera-Johnson, Be.; Thrift-Cahall, E.; Liddick, M.; Tank, J.; Bolster, D.; Bibby, K.

Exploring python scripts in Geographic Information Systems (GIS) to reveal distribution patterns of antimicrobial resistance in the environment
Patrick Spets - Ebert, K.; Dinnétz, P.

Spatial differences in the human gut microbiota selects distinct antimicrobial resistance genes in a simulated in vitro reactor
Fathima Afsal - Swarupha, J.; Frigon, D.; Kubow, S.; Iskander, M.; Gaisawat, M. B.; Lopez Castro, J. S.

Antibiotics and antibiotic resistance genes in an urbanized River
Paola Grenni - Rolando, L.; De Carolis, C.; Narciso, A.; Patrolecco, L.; Rauseo, J.; Spataro, F.; Monostory, K.; khayer, B.; Toro, K.; Vargha, M.; Barra Caracciolo, A.



Tuesday, May 28 - Room Montréal (Mtl) 3-4

Session 10 (13:45-15:30) : AMR Threats in Agriculture

Antimicrobial resistance (AMR) in soil-plant systems
Invited Speaker: Yong-Guan Zhu

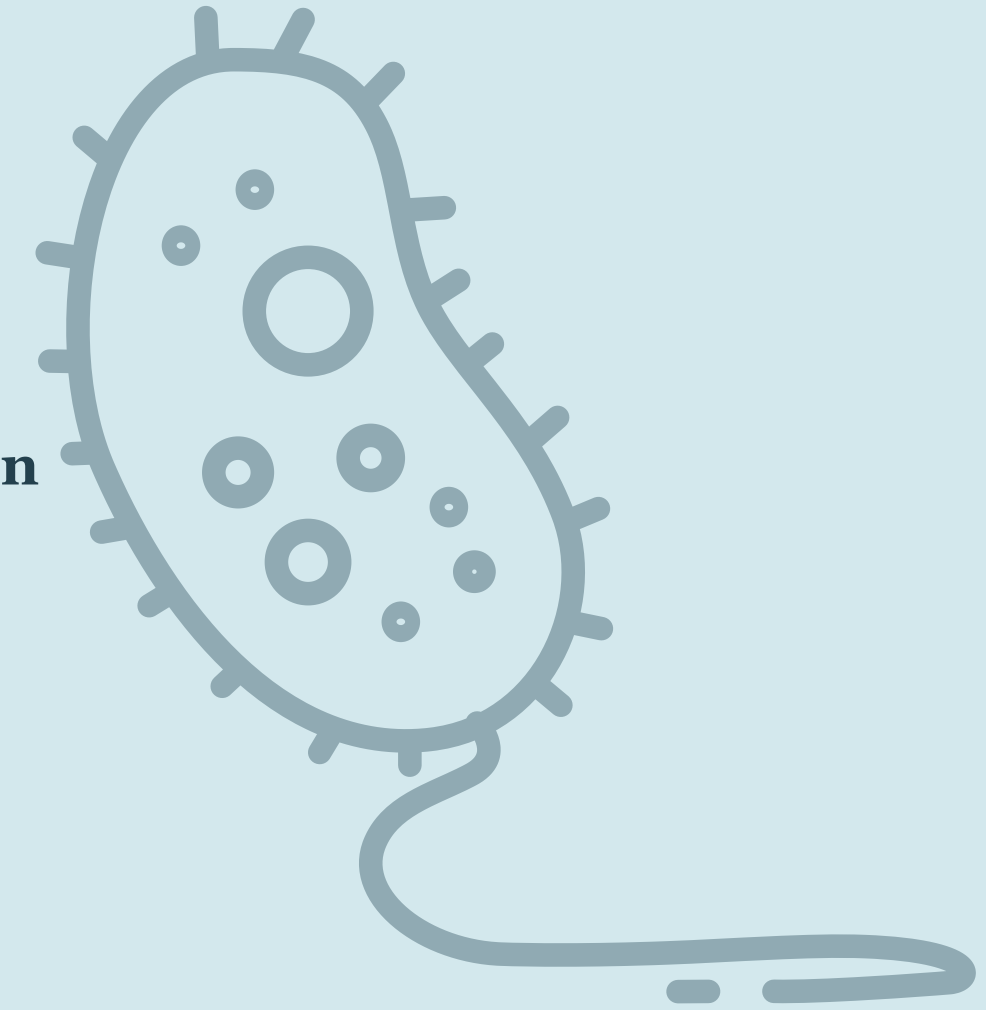
Presentations

Enhancing the Management of Antimicrobial Resistance (EMAR) in Low- and Middle-Income Countries: A Focus on Fiji
Barbara DRIGO - Djordjevic, S. P.; Wyrsch, E.; Okelo, W. O.; Magiri, R.; Lal, V.; Donner, E.

Global distribution pattern, health risks and driving forces of antibiotic resistance genes in livestock manures
Karina Gin - Tiedje, J.; Li, B.; Jiang, L.; Wang, G.

Integrative and conjugative elements harboring antimicrobial resistance in bovine respiratory disease and indicator bacteria
Rahat Zaheer - Andrés-Lasheras, S.; Conrad, C.; Zaidi, S.-E-Z.; In Kim, J.; McAlister, T. A.

From indoor to outdoor bioaerosols: antibiotic-resistance genes and bacterial diversity from pig finishing buildings and broiler operations in Québec
Joanie Lemieux - Dumais, C.; Veillette, M.; Létourneau, V.; Turgeon, N.; Duchaine, C.



Flash Poster Presentations

Impact of Wastewater Discharge on Antibiotic Resistance and Microbial Communities in Fish Guts and Urban Rivers
Molly Mills - Mollenkopf, D.; Wittum, T.; Sullivan, M. P.; Lee, J.

What risk does crop irrigation with reclaimed municipal wastewater represent with respect to the transmission of antimicrobial resistance to humans?
Ed Topp - Alvarez-Fraga, L.; Benoit, P.; Deschamps, M.; Hartmann, A.; Haudin, C.-S.; Hocquet, D.; Le Maréchal, C.; Martin-Laurent, F.; Patureau, D.; Martak, D.

Comparative genomics of Escherichia coli ST131 of human, animal and environmental origin from the Czech Republic
Michaela Ruzickova - Jamborova, I.; Nohejl, T.; Sukkar, I.; Palkovicova, J.; Papousek, I.; Cummins, M.; Djordjevic, S.; Dolejska, M.

Antimicrobial Resistance Profiling of Enteric Bacterial Pathogens in Sheep and Goats at a Livestock Show
Ivan Odur - Pruden, A.; Pithua, P.; Ndegwa, E.; Kim, C.; O'Brien, D.

THE IMPACT OF ANTIBIOTIC GROWTH PROMOTERS (AGPS) ON BROILER CHICKENS' HEALTH AND ENVIRONMENT IN KIBAHA TOWN COUNCIL – TANZANIA
Damas Theobald Msaki

Tuesday, May 28 - Room Montréal (Mtl) 3

Session 11 (13:45-15:30) : Bioinformatics of ARGs

A Standardized Metagenomic Absolute Quantification Method using High-throughput Long-read 3rd Generation Sequencing for Environmental Surveillance of Antibiotic Resistance Genes (ARGs)
Invited Speaker: Tong Zhang

Presentations

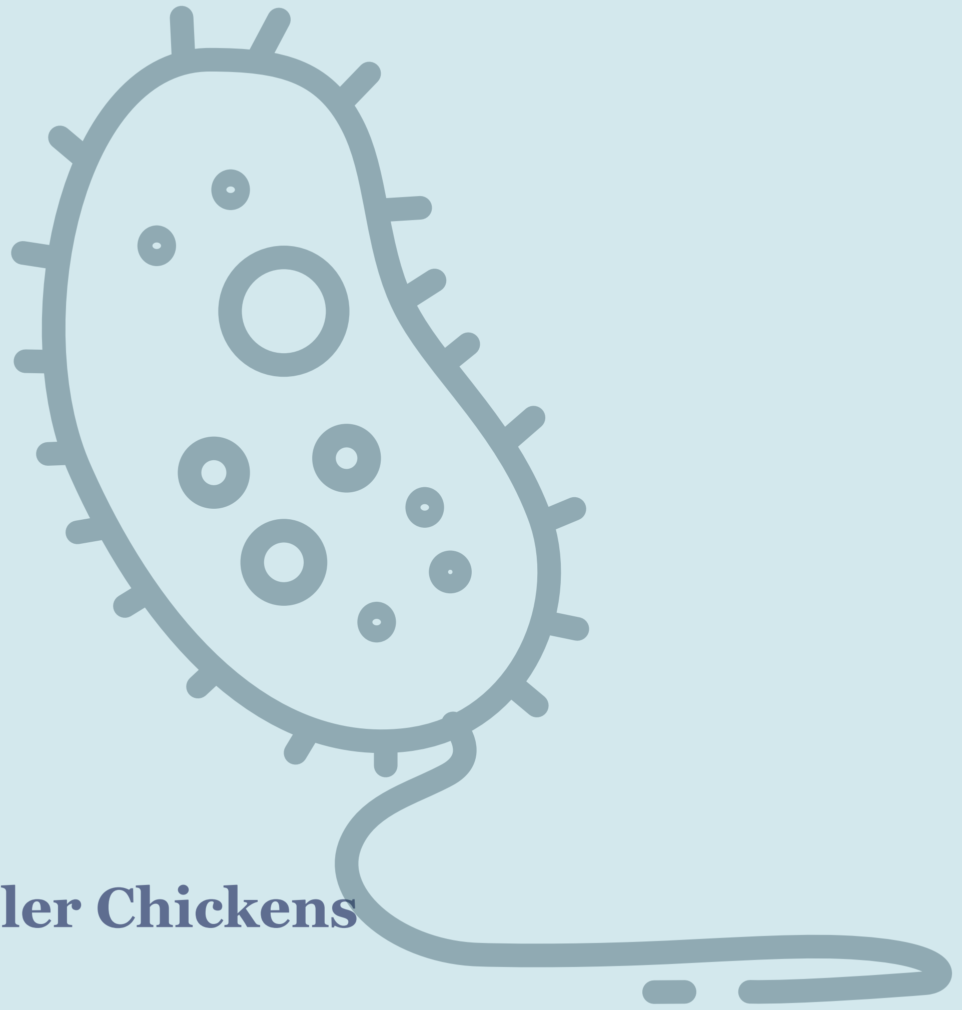
A 3D structure and AI-informed search for the origins and dissemination of sulfonamide resistance genes
Peter Stogios - Venkatesan, M.; Fruci, M.; Savchenko, A.

ResistoXplorer 2.0: an improved bioinformatics tool for comprehensive statistical, visual and integrative analysis of AMR metagenomic sequencing data
Achal Dhariwal - Petersen, F. C.

ESM_2: A Promising Deep Learning Approach For Identifying Novel Antibiotic Resistance Genes in Environmental Metagenomes
Vineeth Manthapuri - Dasu, P. U.; Shukla, A.; Ahmed, S.; Wang, X.; Zhang, L.; Pruden, A.

Harmonizing/integrating surveillance data in the One Health continuum: The GRDI One Health AMR pathogen genomic surveillance contextual data standard
Emma Griffiths - Shay, J.; Sehar, A.; Cameron, R.; Barclay, C.; John, N. S.; Scott, A.; Wajnberg, G.; Robertson, J.s; Schonfeld, J.; Bastedo, P.; Jurga, E.; Johnson, L.; Kess, T.; Nash, J.; Topp, E.; Taboada, E.; Van Domselaar, G.; Smith, D.; Broadbent, J.; Poulin-Laprade, D.; Laing, C.; Zaheer, R.; Reid-Smith, R.; Carrillo, C.; Hsiao, W.

Flash Poster Presentations



Resistance Gene Identifier (RGI) - Prediction of antimicrobial resistance genes in metagenomic sequencing data
Amogelang Raphenya - Alcock, B.; Hackenberger, D.; McArthur, A.

Evaluating a metagenomic workflow for nontargeted detection and quantification of AMR and disease agents in wastewater
Katherine Scott - Brown, C.; Song, H.; Blair, M.; Markham, C.; Davis, B.; Zhang, L.; Vikesland, P.; Pruden, A.

Factors influencing horizontal dissemination of antibiotic resistance genes
David Lund - Parras-Moltó, M.; Inda-Díaz, J. S.; Ebmeyer, S.; Larsson, D. G. J.; Johnning, A.; Kristiansson, E.

Correction effect of Gram positive and Gram negative in extraction and sequencing process based on whole cell spike-in method
Xiang Hui Shi

Genomic Analysis to Determine the Fate of Antimicrobial Resistant Escherichia coli in Soil Amended with Litter from Antibiotic-Fed Broiler Chickens
Paul Waliaula - Islam, R.; Yin, X.; Lepp, D.; Laing, C.; Kiarie, E.; Diarra, M. S.

Tuesday, May 28 - Room Montréal (Mtl) 4

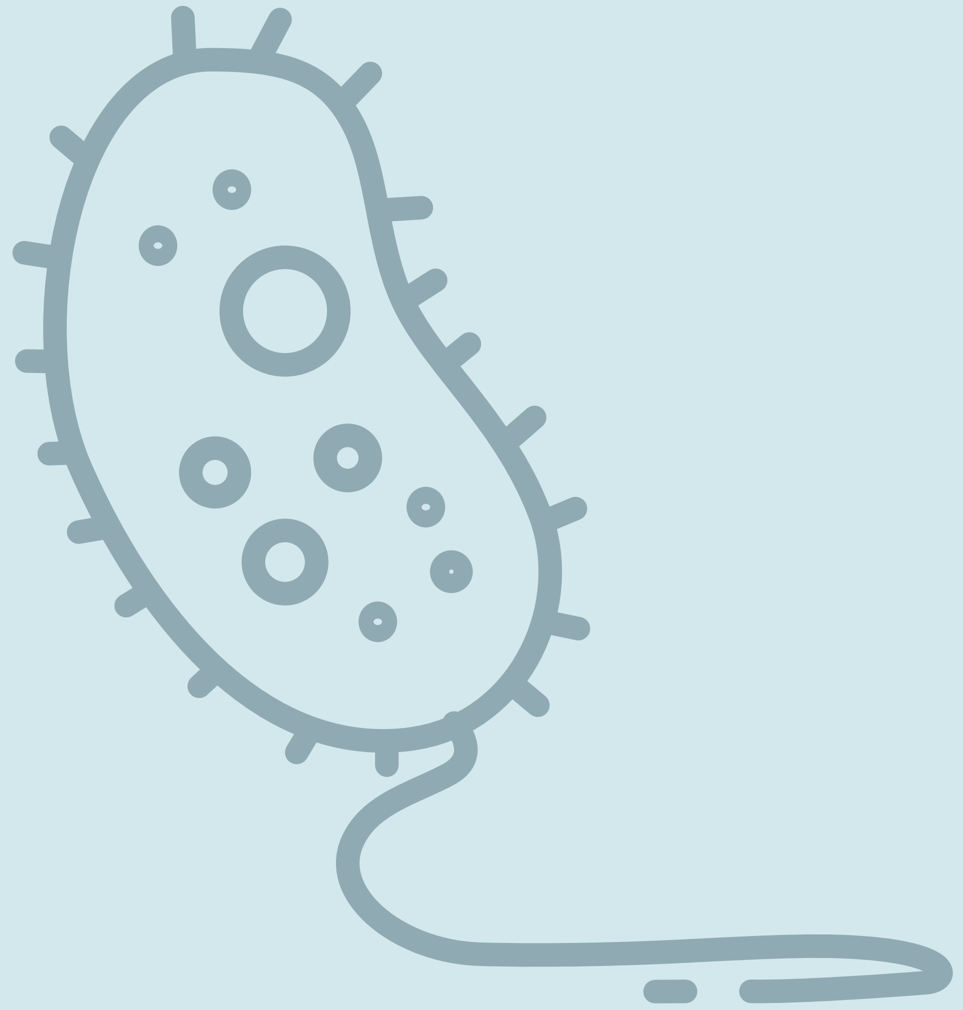
Session 12 (16:00-17:00) : Biosynthesis of Antimicrobials

Production of antimicrobials by Canadian High Arctic bacteria
Invited Speaker: Eszter Farkas

Presentations

Biosynthetic Potential of Antibacterial Compounds Determines Soil Antibiotic Resistomes of Different Land-Use Patterns
Zhiguo Zhang - Ju, F.

Development of control technologies of antibiotics and environmental antibiotic resistance dissemination in pharmaceutical industry
Min Yang - Han, Z.; Wang, C.; Feng, H.; Han, Z.; Liu, S.; Dai, S.; Zhang, Y.



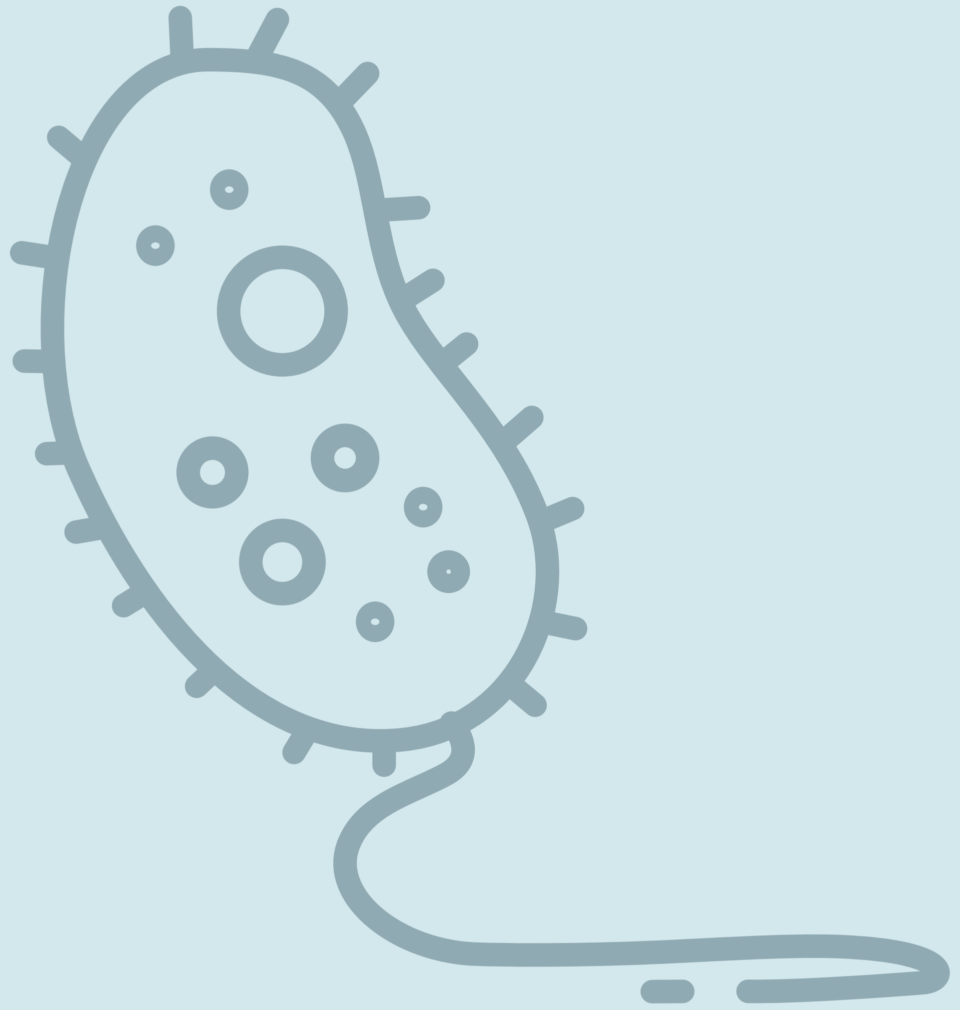
Tuesday, May 28 - Room Montréal (Mtl) 3

Session 13 (16:00-17:00) : Resistance to antifunglas and other antimicrobials

Population genomic approaches to unravelling evolution of drug resistance in pathogenic fungi
Invited Speaker: Johanna Rhodes

Presentations

Citizen scientist surveillance of azole-resistant Aspergillus fumigatus in UK airs and soils
Jennifer Shelton - Stanton, I.; Payne, R.; Rhodes, J.; Uzzell, C.; Hemmings, S.; Brackin, A.; Sewell, T.; Dyer, P.; Alghamdi, A.; Borman, A.; Johnson, E.; Piel, F.; Singer, A.; Fisher, M.



Wednesday, May 29 - Room Montréal (Mtl) 3-4

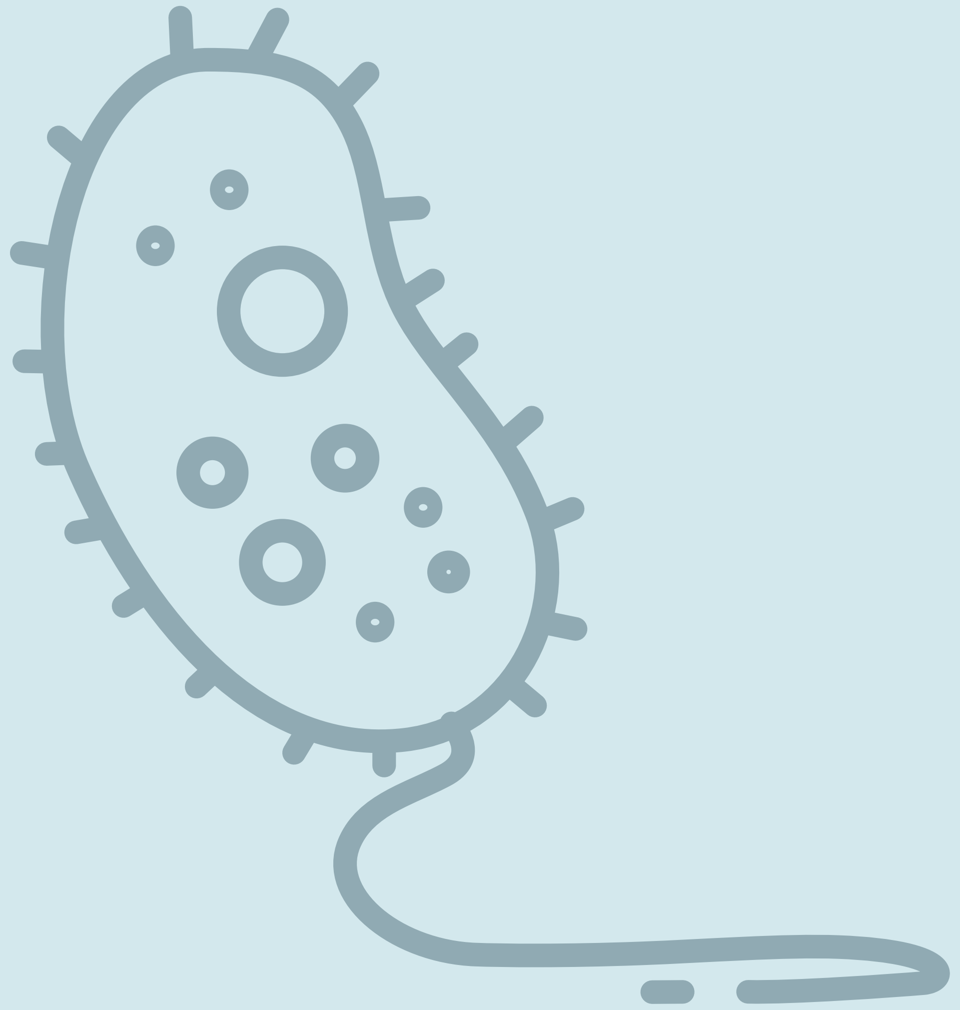
Session 14 (8:30-9:30) : Intergrated surveillance along the One Health continuum

What does integration look like? - One Health-thinking, harmonised methods, and global context
Invited Speaker: David W Graham

Presentations

One Health, One Data: Describing Antimicrobial Resistance in Surface Water in the United States
Andrea Ottesen - Kocurek, B.; McDermott, P.; Strain, E.

Evidence-Integrated Insights on Antimicrobial Resistance for better understanding "One-Health"
Cai Chen - Li, S.; Zhu, Y.-G.; Graham, D.



Wednesday, May 29 - Room Montréal (Mtl) 3-4

Session 15 (9:30-10:30) - Technical Exhibitor Session

Resistomap Intelligence Platform for environmental AMR monitoring
Windi Muziasari - Resistomap

Minimizing Bias in Microbial Research
Bylan Barbera - Qiagen Inc

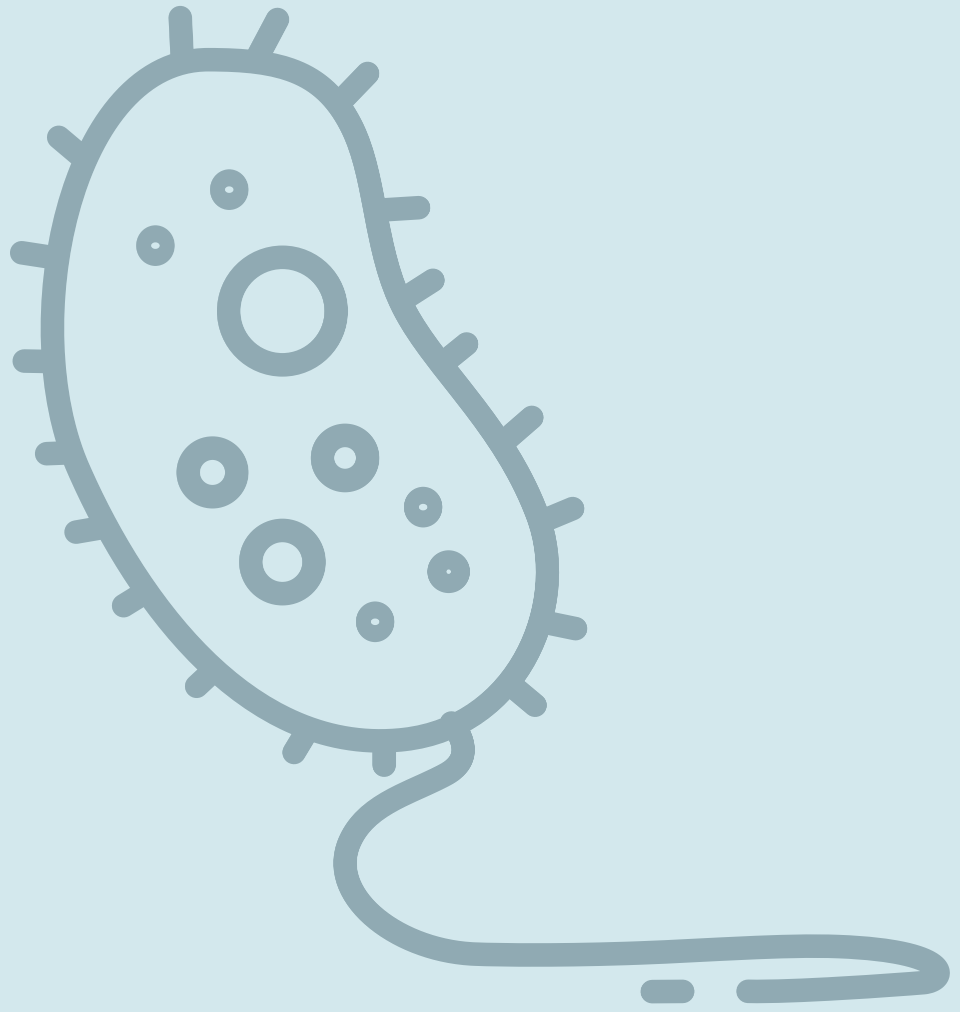
Illumina's XLEAP Chemistry Unlocks Accessibility to Highly Sensitive AMR Detection
Jeffrey Gross - Illumina ULC

One Health Panel Discussion (11:15-12:00)

By Genome Quebec & Public Health Agency of Canada (PHAC)

Pre-Workshop Communication (12:00-12:30)

By Dr. Chantal Morel (WHO - Quadripartite Secretariat)
Scientific Consultation of the Quadripartite ahead of the 2024 UN General Assembly High-level Meeting on AMR



Workshops

1 - EDAR in Asia-Pacific LMICs – Research needs and Regional Guideline for AMR surveillance in the Animal Farm Environment - Zaheer, Honda

2 - Strengthening the Policy Relevance of Wastewater-based Surveillance for AMR - Conforti, Pruden

3 - Exploring the Factors Influencing Antibiotic Resistance Beyond the Wastewater Pipeline - Manaia, Berendonk

4 - Mutual understanding between regulatory needs and science-based risk assessment of environmental AMR - Sander, Schreiber

5 - Navigating the Labyrinth Integrating the Environment into AMR Action Plans - Musmar, Francis

6 - Gaming AMR Role-Play Simulation Exploring the Policy Dimensions of AMR - Riddle, Schenk, Kassen

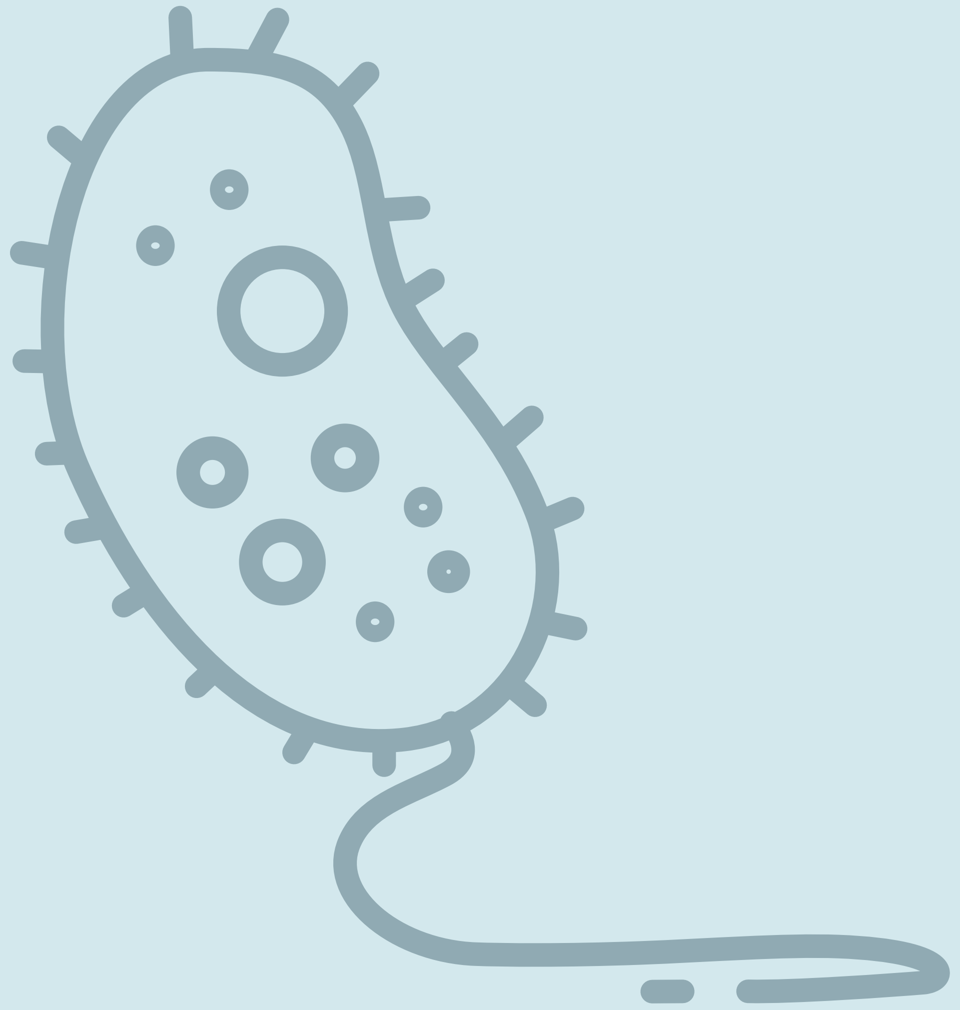
7 - Detection of AMR in managed agricultural environments - Yang, Zhao

8 - Integration of genomics data, risk science, and practical considerations - Broadbent, Poulin-Laprade

9 - So you have AMR data, now what Applications for actionable risk assessment and decision-making - Hamilton

10 - Resistomap Intelligence Platform for environmental AMR monitoring (open) - Muziasari, Seppala

11 - Quadripartite workshop for EDAR - Morel, Topp



Thursday, May 30 - Room Montréal (Mtl) 3-4

Session 16 (8:30-10:15) : One Health AMR biosecurity and mitigation approaches

Quantifying the global impact and inequities associated with food-animal E. coli
Invited Speaker: Lance B. Price

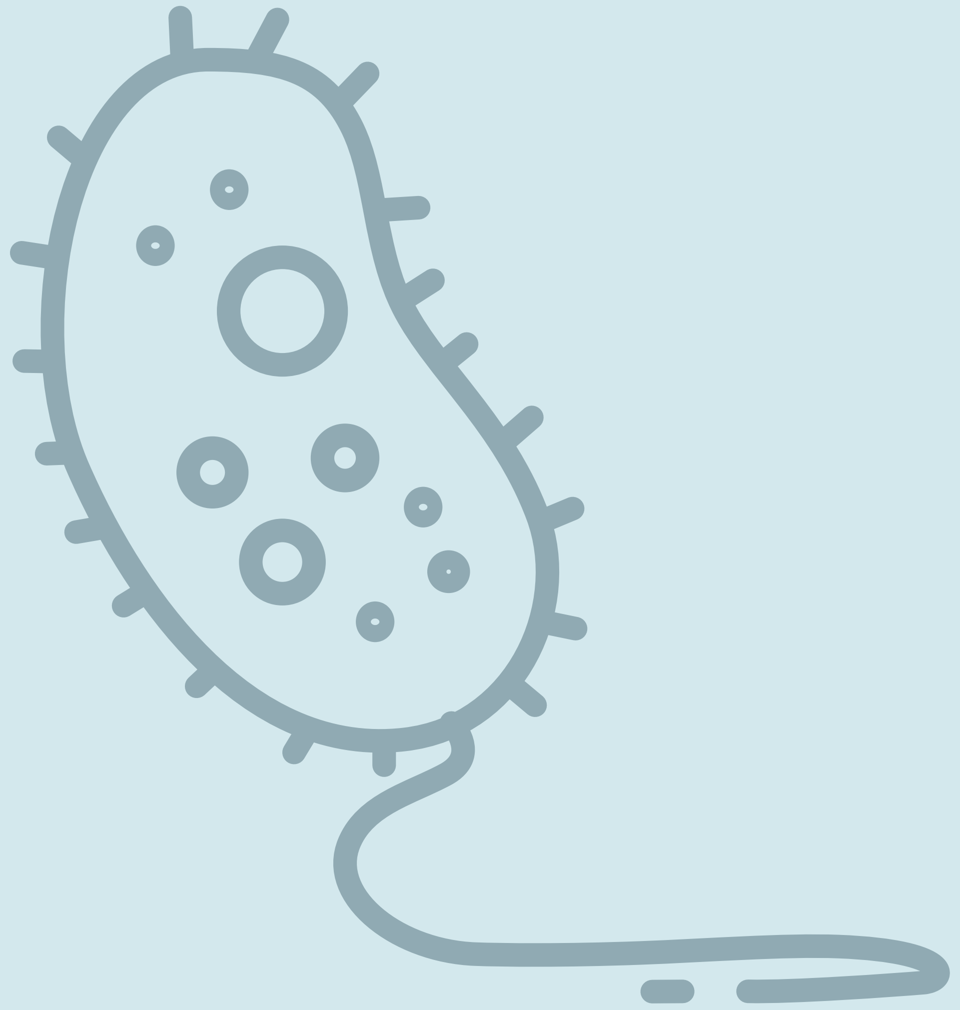
Presentations

Antibiotic ecotoxicity and resistance risks in chicken and pig farming environments in Kenya
Fredrick Gudda - Moodley, A.; Muloi, D.; Gao, Y.

Strategies to Combat Antibiotic Resistance Genes Using Magnetic Biochar/Quaternary Phosphonium Salt in Agricultural Systems
Fang Wang

Constructed wetlands as a nature-based solution for removing antibiotics and antibiotic resistance genes from wastewater
Anna Barra Caracciolo

Environmental antimicrobial resistance threats to food safety and security
Kiranmayee Bhimavarapu - Huber, L.; Williams, G.; Rodrigues, C.; da Silva, A. L.; Boersma, M.



Flash Poster Presentations

Improving the DARTE-QM Monitoring Platform for a One-Health Surveillance Approach
Daniel Gyamfi Amoako - Alcock, B.; McArthur, A.; Ricker, N.

Monitoring the potential escape of bioaerosolised antimicrobial resistance genes from wastewater treatment plants with air and phyllosphere sampling
Paul George - Hillary, L.; Leclerc, S.; Cooledge, E.; Lemieux, J.; Duchaine, C.; Jones, D.

Seeds act as vectors for antibiotic resistance gene dissemination in a soil-plant continuum
Xin-Yuan Li - Wu, W.-F.; Lin, X.-Y.; Zhu, Y.-G.

Genomic analysis of ESBL-producing bacteria recovered from frozen, ready-to-eat, imported stone fruit
Catherine Carrillo - Adeeb, N.; Cooper, A.; Tamber, S.

Thursday, May 30 - Room Montréal (Mtl) 4

Session 17 (11:00-12:30) : Environmental Polution Impact on AMR Development and Its Surveillance

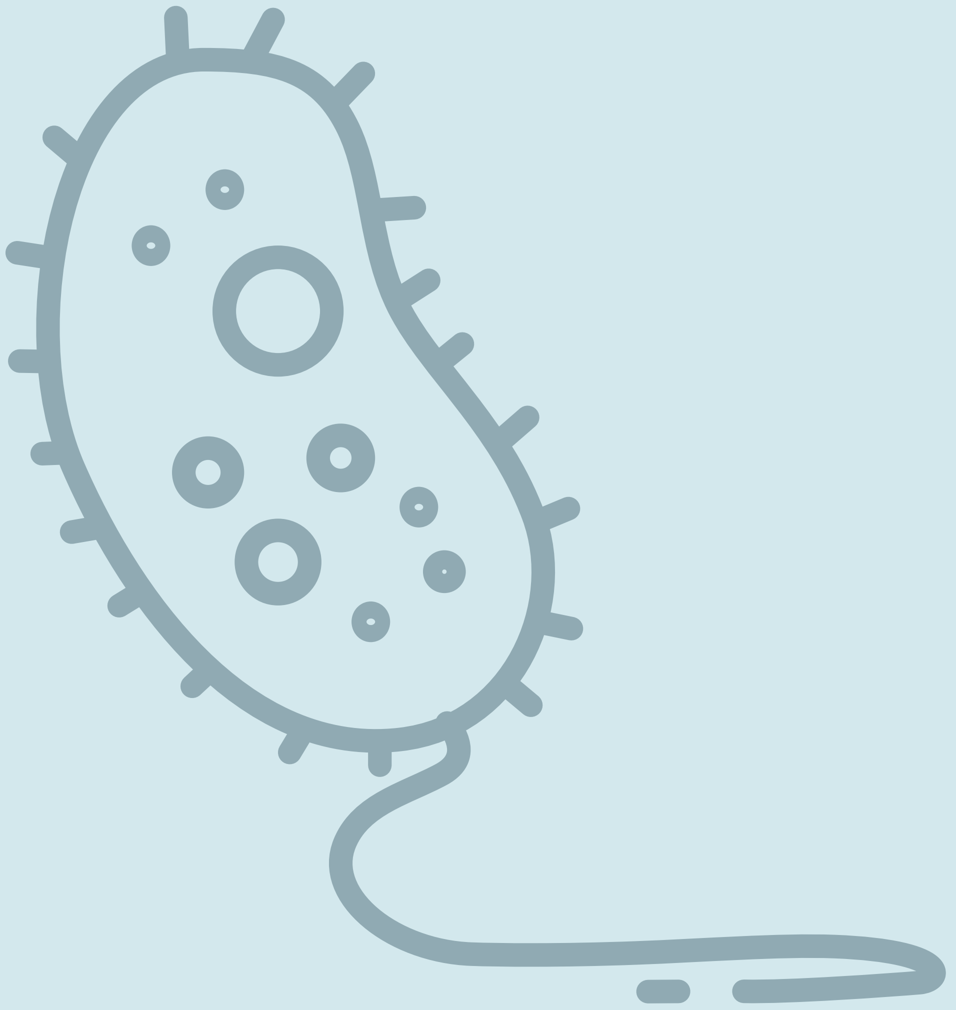
AMR and pathogen evolution in a One Health context
Invited Speaker: Steven Djordjevic

Presentations

Dangerous hitchhikers! - How cigarette filters promote the spread of antimicrobial resistant bacteria in the environment.
Uli Klümper - Fang, P.; Konyali, D.; Li, B.; Dalpke, A.; Huang, J.; Berendonk, T.

The impact of various microplastics on bacterial community and antimicrobial resistance genes in Norwegian and South African wastewater
Tam Thanh Tran - Stenger, K. S.; Bezuidenhout, C. C.; Strømmen, M.; Wikmark, O.-G.

Cyanobacterial Blooms are Antibiotic-Resistant Gene Reservoirs in Drinking Water Sources: Potential Public Health Impacts Beyond Cyanotoxins
Jiyoung Lee



Flash Poster Presentations

Impacts of Antibiotic Pesticide Use in Crop Agriculture on Antibiotic Resistance in Human Pathogens and Commensals: A Systematic Review
Mary Schoen - Jahne, M.; Garland, J.

Assessing the impact of anthropogenic pollution on microbial diversity and AMR in recreational waters using metagenomic sequencing
Dearbháile Morris - Cahill, N.; Miliotis, G.; O'Connor, L.; Hooban, B.

Heavy metals increase antibiotic resistance gene maintenance in the environment
Emilie Dehon - Mathieu, A.; Droit, A.; Vogel, T. M.; Sanchez-Cid, C.

Methane and Nitrous Oxide Emissions from Manure: Effects of Antibiotic Residues
Chebet Arusey - Moodley, A.; Leitner, S.; Muloi, D.; Ng'etich, W.; Kebeney, S.; Yuhao, Z.; Oduor, C.

The effect of propidium monoazide (PMA) pre-treatment on qPCR-based pathogen survival monitoring of ozone-treated wastewater effluent
Elfy Ly - van de Pol, D.; van Loosdrecht, M.; Hernández Leal, L.; Schmitt, H.; van Veelen, P.

Thursday, May 30 - Room Montréal (Mtl) 3

Session 18 (11:00-12:30) : Waterwaterbased surveillance and link to human populations

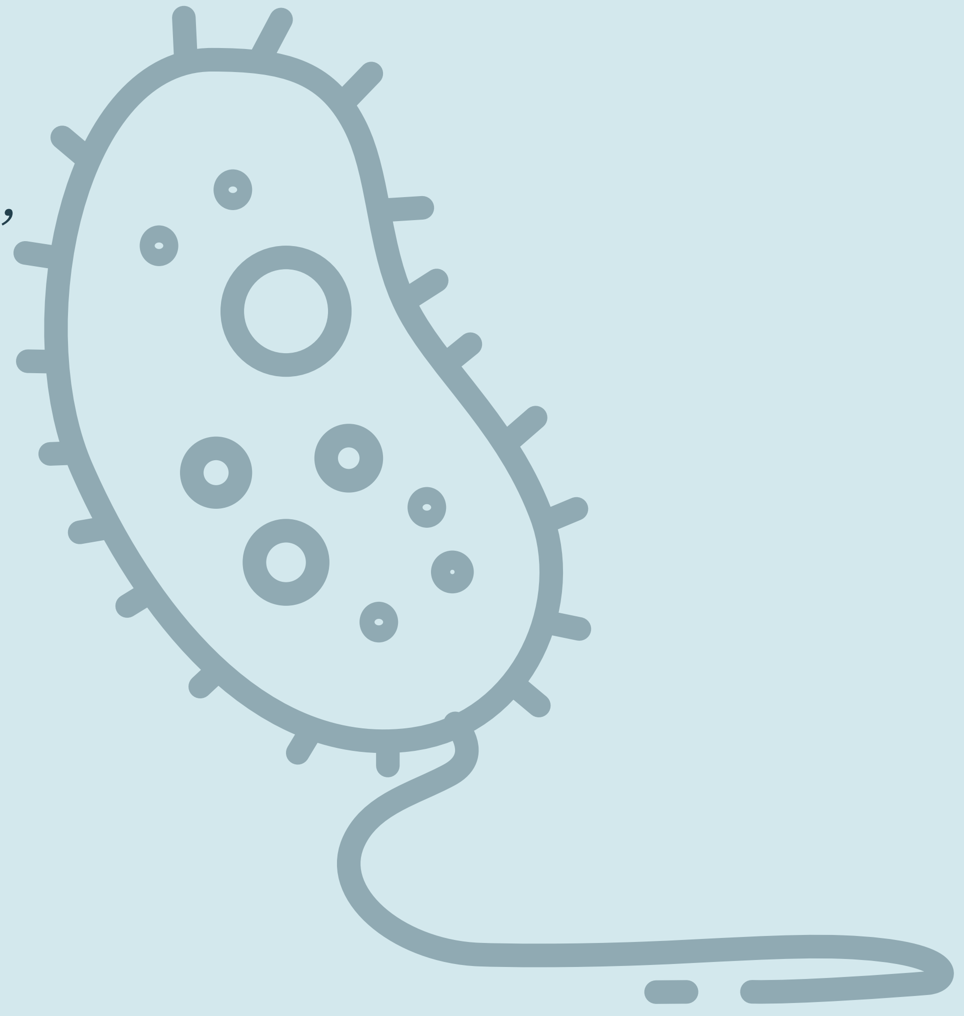
Concentrations in WWTP influents mirror fecal discharge and community carriage of resistant bacteria
Invited Speaker: Heike Schmitt

Presentations

Resistance in sewage-associated microbiota mirror patterns of clinical isolates and reflect a variable lagging association with antibiotic usage
Connor Brown - Rumi, M. A.; McDaniel, L.; Maile-Moskowitz, A.; Sein, J.; Choi, M.; Hindi, F.; Emon, M.; Ahmad Moumi, N.; Blair, M.; Rao, J.; Baffoe-Bonnie, A.; Vikesland, P.; Pruden, A.; Zhang, L.

Culture enriched wastewater exhibits an increased burden of antibiotic resistant genes in hospitals relative to the community
Nicole Acosta - Lee, J.; Bautista, M. A.; Bhatnagar, S.; Waddell, B. J.; Au, E.; Pradhan, P.; Clark, R. G.; Meddings, J.; Ruecker, N. J.; Achari, G.; Pitout, J.; Conly, J.; Frankowski, K.; Hubert, C.; Parkins, M. D.

From clinics to sewers: leveraging environmental surveillance to inform epidemiology of clinically-relevant antibiotic resistant bacteria
Sheena Conforti - Holschneider, A.; Habluetzel, C.; Sylvestre, É.; Du Plessis, L.; Stadler, T.; Julian, T. R.



Flash Poster Presentations

Association of population's lifestyles and behaviours with the prevalence of antimicrobial resistance in municipal wastewater
Helena Leal - Klimova, N.; Gibson, C.; Fortin, É.; He, J.; Frigon, D.; Dorner, S.; Quach, C.; Bédard, É.

Considerations for Wastewater-Based Epidemiology of AMR in Communities with Compromised Sewage Collection Networks
Amanda Darling - Deck, M.; Byrne, T.; Maldonado Rivera, G.; Price, S.; Amaral-Torres, A.; Markham, C.; Davis, B.; Vikesland, P.; Krometis, L.-A.; Cohen, A.; Pruden, A.

Sewage surveillance of antibiotic resistance - possibilities, limitations and pitfalls
Carl-Fredrik Flach

Longitudinal ARGs Profiles in Wastewater Treatment Plant Influent Revealed by Metagenomic Analysis
Shuxian LI - ZHANG, T.

Carbapenemase gene blaOXA-48 detected at six freshwater sites in Northern Ireland discharging onto identified bathing locations
Cathy Brooks - Mitchell, E.; Brown, J.; Carnaghan, K.-A.; Bleakney, E.; Arnscheidt, J.

Thursday, May 30 - Room Montréal (Mtl) 4

Session 19 (13:45-15:30) : Wildlife and other environmental reservoirs of AMR

Changing dynamics of antibiotic resistant Escherichia spp. in Caspian gulls shows the importance of longitudinal environmental studies
Invited Speaker: Monika Dolejska

Presentations

Evils and allies: gulls as spreaders and sentinels of Antibiotic Resistant Bacteria in human-transformed environments
Martín Vélez; Montalvo, T.; Gimenez, J.; Ramirez, F.; Sarda-Palomera, F.; Santisteban, C.; Sabate, S.; Planell, R.; Morral-Puigmal, C.; Giralt, D.; Bota, G.; Navarro, J.

Zooplankton affects extracellular DNA degradation and uptake by natural transformation in freshwater microcosms
Elena Crotti - Riva, F.; Ferrari, E.; Lazzaro, F.; Borin, S.; Di Cesare, A.; Mapelli, F.; Eckert, E.

Impact of wastewater effluents on the carriage of antibiotic resistant bacteria and genes in cattle grazing downstream wastewater treatment plants
María Rincón - Söderlund, R.; Rosendal, T.; Bonnedahl, J.; Woksepp, H.; Börjesson, S.; Hernández, J.; Green, A. J.; Martín-Vélez, V.; Navarro-Ramos, M. J.

Hosts of antibiotic-resistance genes from Croatian wastewaters
Sujetlana Dekic Rozman - Puljko, A.; Karkman, A.; Virta, M.; Udikovic-Kolic, N.

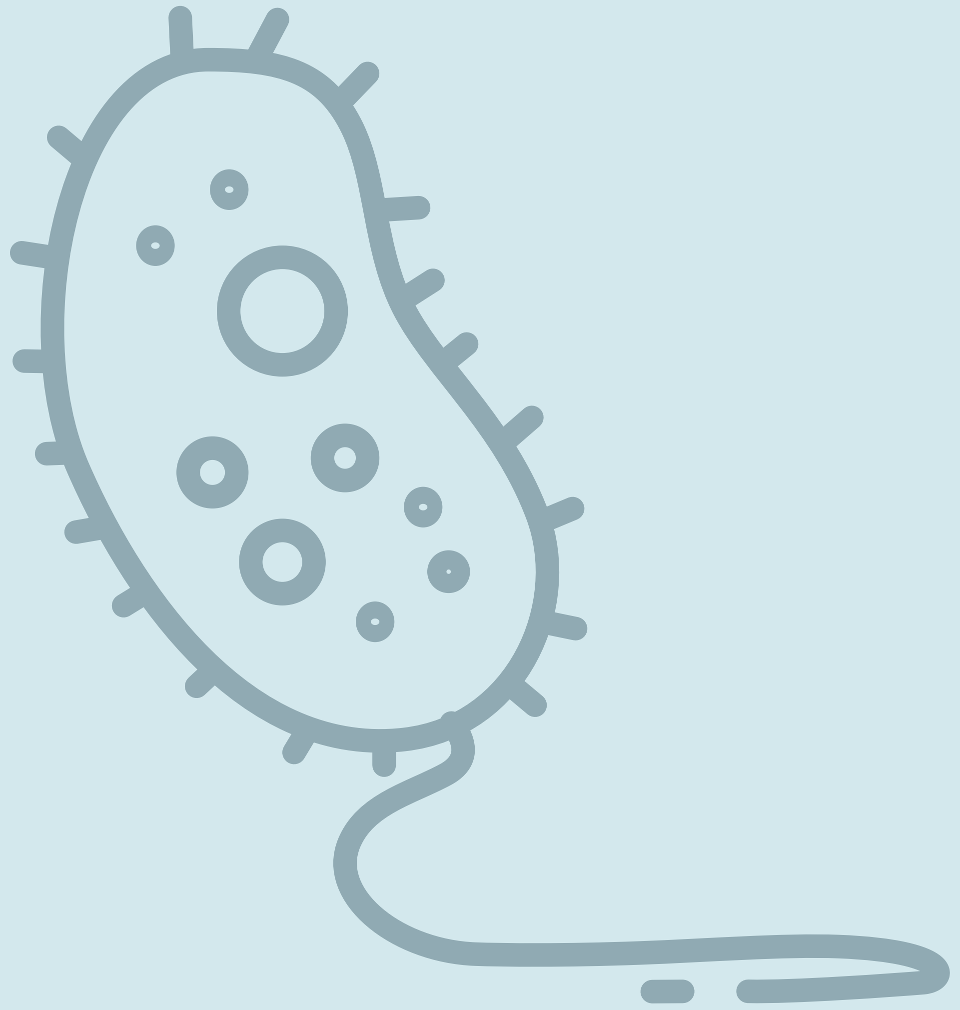
Flash Poster Presentations

The role of synanthropic birds as sources and potential transmission agents of AMR in Uganda
Charles Masembe, Odong, Robinson - Mayega, J. F.; Akoll, P.; Omara, J.; Katende, G.; Ndinawe, R. P.

Current knowledge of AMR in wild fauna and flora
Rachel Payne - Tipper, H.; Stanton, I.; Shelton, J.; Singer, A.; Read, D.

The co-occurrence and potential transfer risks of antibiotic resistomes between non-intensive aquaculture environment and human
Yanping Mao - Tian, L.

Microbial risks triggered through oral administration of antibiotics in fish aquaculture far persist the legally regulated antibiotic withdrawal time
Bing Li



Thursday, May 30 - Room Montréal (Mtl) 3

Session 20 (13:45-15:30) - Predicted No-Effect Concentration of Antimicrobials

Investigating AMR evolution in the environment
Invited Speaker: William Gaze

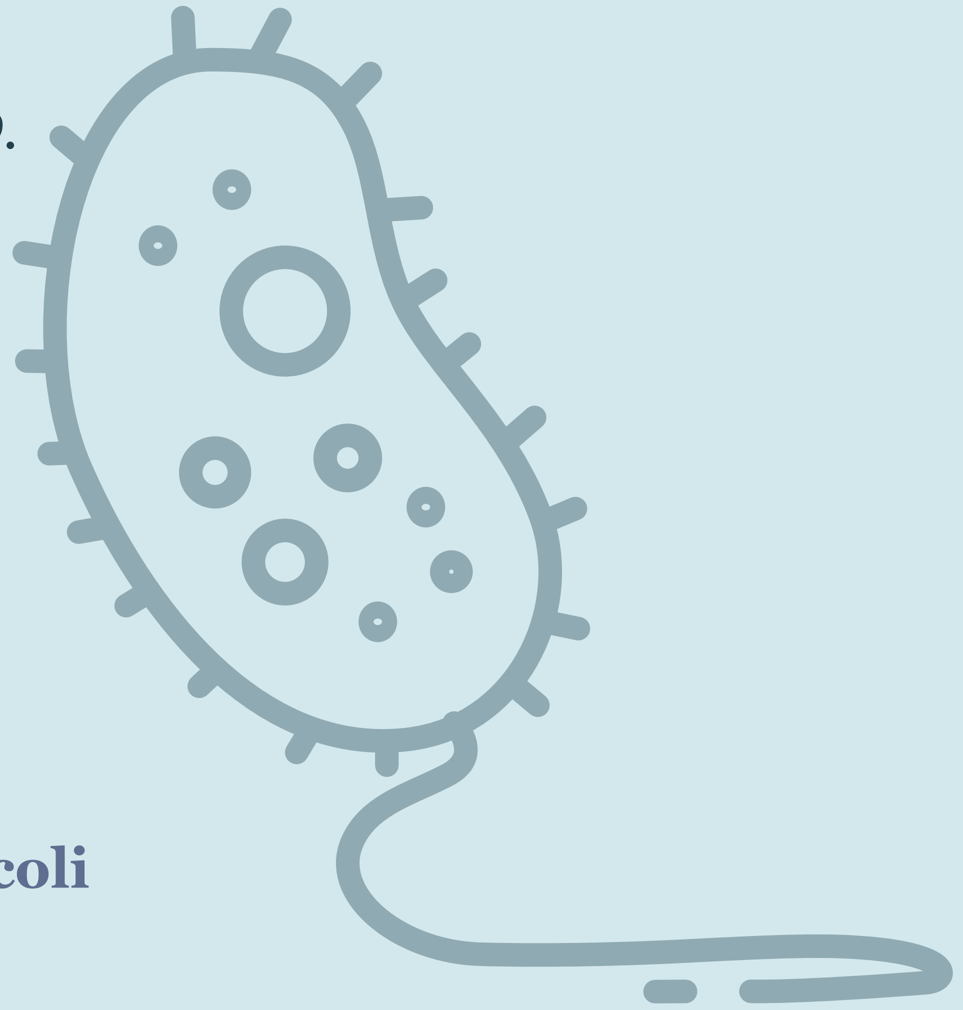
◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇ **Presentations** ◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇

Effect of pharmaceutical manufacturing waste on the environmental resistome: are there implications for human health?
Chiara Borsetto - Travis, E.; Raguideau, S.; Bhatt, A.; Maharana, B.; Dafale, N.; Quince, C.; Song, L.; Taneja, N.; Wellington, E.

Evolution of antibiotic resistance in soils in presence of increased concentrations of sulfamethoxazole and clarithromycin
Oriane Della-Negra - Vittoria Barbieri, M.; Camotti Bastos, M.; Bru-Adan, V.; Santa-Catalina, G.; Ait Mouheb, N.; Heran, M.; Wéry, N.; Chiron, S.; Patureau, D.

European Union regulatory measures for the reduction of antimicrobial resistance in the environment - Oversight on the implementation of EU RL 2019/6
Thomas Heberer - Sander, S.

Antibiotic Manufacturing Standard: An industry approach for responsible effluent management in the context of antibacterial resistance
Andreas Häner - Elmoznino, J.; Reid, K.; Brooks, S.



◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇ **Flash Poster Presentations** ◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇◇

Pharmaceuticals at concentrations exceeding PNEC values in the aquatic environment fail to induce antimicrobial resistance in Escherichia coli
Luther King Abia Akebe - Chukwu, K. B.; Abafe, O. A.; Amoako, D. G.; Arshad, I.; Essack, S. Y.

The Minimum Ciprofloxacin Concentration Affecting the Microbial Community and Antimicrobial Resistance Gene Compositions of Anaerobic Processes
Sukriye Celikkol - Lafaille, M; Kramer, S.; Caldwell, C.; Dinh, Q. T.; Hassan, Z.; Sauvé, S.; Frigon, D.; Vo Duy, S.

Strain-level diversity in sulfonamide biodegradation: adaptation of Paenarthrobacter to sulfonamides
Yue Huang - Zhang, T.

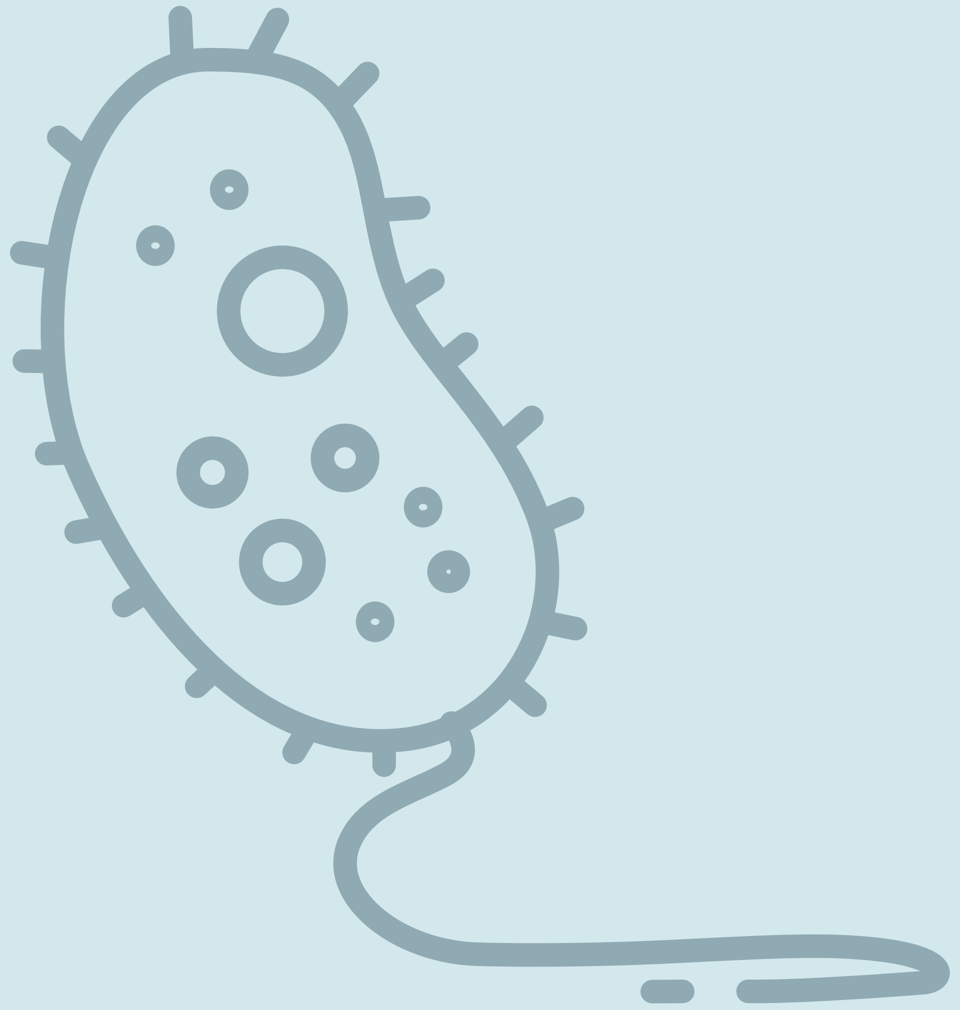
Prevalence and Antimicrobial Resistance Profile of Bacterial Isolates from the Environment of Higher Education Institutions in Maputo, Mozambique
Cristiano Macuamule - Raice, F.

The ARISE project: Pioneering wastewater-based AMR surveillance in alignment with upcoming EU regulatory changes
Claudia Kolm - Vierheilig, J.; Kreuzinger, N.; Zarfel, G.; Ströbele, B.; Finsterwald, M.; Zepke, G.; Martzy, R.; Stelzer, T.; Weinberger, J.; Strauß, R.; Lehner, A.; Weber, J.; Müller-Rechberger, H.; Nykyforuk, L.; Kirschner, A.; Farnleitner, A.

Friday, May 31 - Room Montréal (Mtl) 3-4

Session 21 (8:30 - 9:00)

Taking a Systems Approach to Addressing the Environmental Dimensions of Antimicrobial Resistance
Invited Speaker: Anthony So



Sunday, May 26 - 16:00-17:00

Back to the Program

Keynote Speaker: Gerry Wright

Session 1
Abstract 419
Room Mtl 3-4

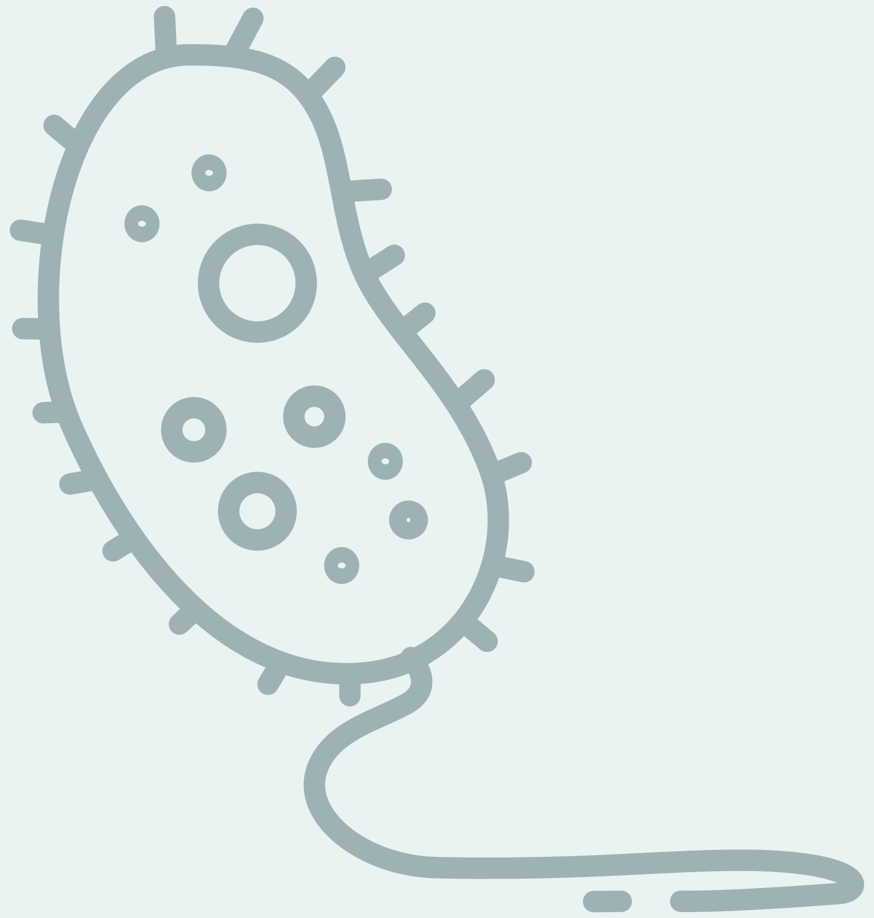
The Natural History of Antibiotic Resistance

Resistance to antibiotics has emerged as a threat to modern medicine and agriculture across the globe. The study of resistance is understandably focused on the development of resistance in infectious organisms that previously were antibiotic susceptible. However, a more comprehensive understanding of resistance would include recognizing the long history of microorganisms on the planet, the interaction between other organisms and the environment, and the evolution and behavior of microorganisms in the face of myriad challenges. When viewed through this lens, antibiotic resistance emerges as a natural phenomenon with extensive roots in microbial genomes that extend deep into the past. By exploring the diversity and evolution of contemporary microorganisms and historical samples, we can retrace the origins of resistance, anticipate the emergence of new resistance elements, and explore the broad diversity of molecular mechanisms and their associated genetic elements that make up the antibiotic resistome.

Author: Gerry Wright, Michael G. DeGroote Institute for Infectious Disease Research, Canada

Biography:

Gerard (Gerry) Wright is the Michael G. DeGroote Chair in Infection and Anti-Infective Research and Professor in the Department of Biochemistry and Biomedical Science at McMaster University, Hamilton, Ontario. He was the founding director of the Michael G. DeGroote Institute for Infectious Disease Research (2007-2022), the David Braley Centre for Antibiotic Discovery (2018-2022), and McMaster's Global Nexus School for Pandemic Prevention and Response (2020-2023). Gerry was elected as a Fellow of the Royal Society of Canada (2012) and a fellow of the American Academy of Microbiology (2013). He has trained over 90 graduate students and postdoctoral fellows and is the author of over 315 manuscripts. His research interests are in the origins and mechanisms of antibiotic resistance and the discovery of new anti-infective strategies, particularly focusing on applying microbial natural products and synthetic biology to this goal.



Back to the Program

Session 2
Abstract 220
Room 3-4

Where do antibiotic resistance genes become mobile?

Background and aim: Understanding in which environments chromosomal antibiotic resistance genes (ARGs) acquire increased mobility is crucial to effectively mitigate future emergence of additional ARGs in pathogens. One way could be to learn from history and identify the bacteria that are the chromosomal origins of already mobilised ARGs, and to map the habitats of those. Previously, we have defined criteria and verified origins of ca 30 ARGs. Most origin species were common in wastewaters, but rare in human microbiota. Still, origins for most mobile ARGs remain unknown. Therefore, a main aim was to develop and apply a large-scale approach for origin species identification, map their habitats, and compare these with respect to prevailing selection pressures, including new data on various wastewaters.

Method: Data from >1.5 million genome assemblies were used to train a random forest classifier to identify the chromosomal origins of mobile ARGs. An optimized Kraken2 database was generated to map their distribution using public metagenomic data. Selection experiments with complex wastewaters were performed to better understand evolutionary drivers.

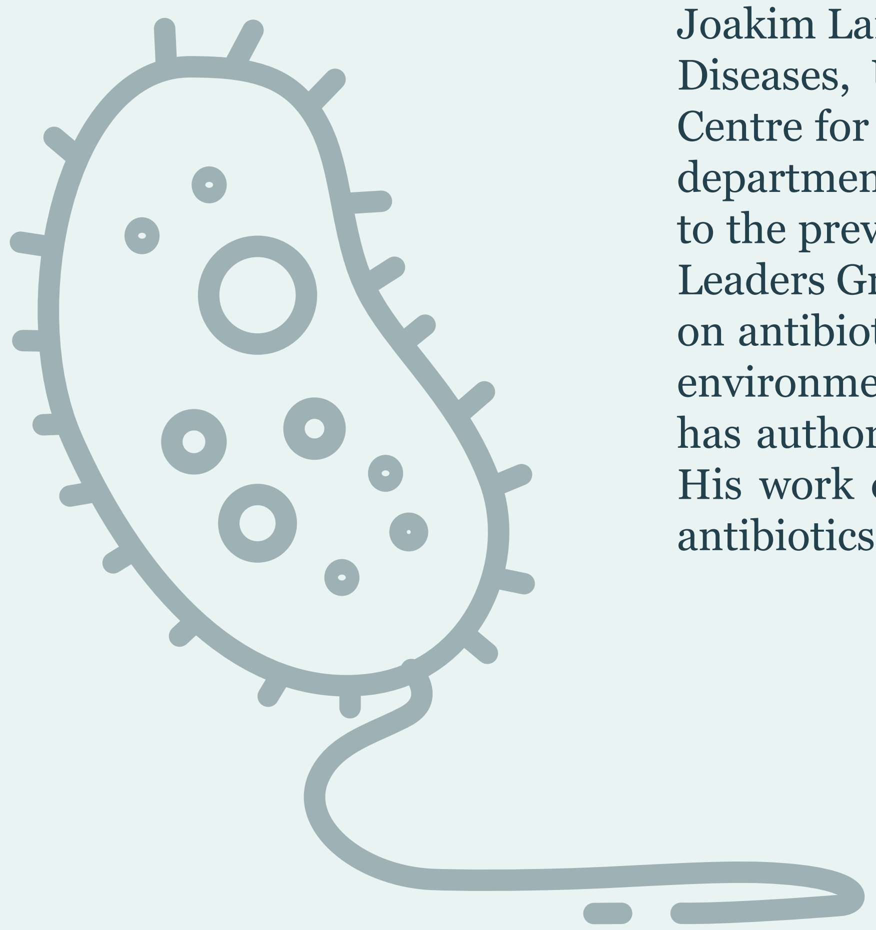
Findings: We identified ten previously unknown origins of mobile ARG groups, including the first strong evidence for origins of tetracycline resistance genes. These latter species were particularly common in animal feces, in agreement with exceptionally high use of tetracyclines in animal production. Often, multiple ARGs came from the same species, suggesting favourable genetic/ecological traits. Most origin species were particularly abundant in municipal wastewaters, while three were most common in environments polluted with waste from antibiotic manufacturing. Selection experiments with wastewaters showed, as expected, a clear gradient from industrial, hospital to municipal sources, with less evidence for strong selection in treated municipal effluents.

Implications: This study highlights environments where origin species of ARGs thrive and where there is an apparent need for limiting antibiotic selection pressures to mitigate future mobilisation events.

Author: D. G. Joakim Larsson, University of Gothenburg, Sweden

Biography:

Joakim Larsson is a Professor in Environmental Pharmacology at the Department of Infectious Diseases, University of Gothenburg, Sweden, where he is director for the multidisciplinary Centre for Antibiotic Resistance Research (CARe) in Gothenburg with ca 155 members from 18 departments. He is a scientific advisory board member of the JPIAMR, and personal advisor to the previous and current Swedish Ministers of Health in their role as members of the Global Leaders Group on AMR. He also works as consultant for the WHO on an international guidance on antibiotic pollution from manufacturing, to be released in 2024. His own research focus on environmental dimensions of antibiotic resistance, particularly evolution and surveillance. He has authored +200 papers and he is on Clarivate's list of highly cited researchers since 2018. His work on pollution from drug manufacturing and research on selective concentrations of antibiotics has contributed to various management initiatives across the world.



Session 2
Abstract 60
Room 3-4

Prevalence and transmission of antibiotic resistance in wastewater treatment systems

Background and Aim: Antibiotic resistance in wastewater treatment systems is a growing concern due to its potential impact on public health and the environment. Numerous studies have investigated the distribution and types of antibiotic resistance genes (ARGs) in various biological wastewater treatment systems. However, limited information is available on their prevalence and transmission throughout the entire treatment process and their host sources. Therefore, this study aims to determine the prevalence of ARGs in influent, activated sludge, effluent, and anaerobic digestion sludge, as well as identify their host sources.

Procedure/Method: In this study, samples representing the whole wastewater treatment process, including influent, activated sludge, effluent, and anaerobic digestion sludge, were collected from six wastewater treatment plants. Metagenomic and Hi-C sequencing techniques were employed to reconstruct and retrieve prokaryotic genomes, phage genomes, plasmid sequences, and ARGs, as well as to establish the relationship between these genetic elements.

Findings/Results: The highest abundance of ARGs was detected in influent samples, while the prevalence in the other three sample types was significantly lower and comparable. In agreement with the prevalence of ARGs in influent, numerous plasmids were also identified in influent samples. Furthermore, Hi-C results demonstrated that the majority of ARGs originated from genomic and plasmid sources, which explained the consistent trends of plasmids and ARGs in these samples. Although a high prevalence of phages was observed, particularly in effluent samples, none of the ARGs were associated with these phages. This finding underscores the critical role of plasmids in the transmission of antibiotic resistance within wastewater treatment systems.

Implications/Applications: Our study offers valuable insights into the prevalence of ARGs throughout the wastewater treatment process and confirms the primary host sources of ARGs.

Author: Dou Wang, The University of Hong Kong, China

Co-Author: Zhang, Tong

Monday, May 27 - 8:30-10:15

Back to the Program

Session 2
Abstract 292
Room 3-4

Genomic disparities in extended-spectrum β-lactamase-selected versus non-selected Escherichia coli from Australian wastewater

Background: ESBL-producing E. coli, resistant to most β-lactam antibiotics, necessitate “last-resort” antibiotics. The Tricycle protocol describes a OneHealth AMR surveillance system with ESBL-producing E. coli as its singular indicator organism. However, exclusive focus on ESBL-producing strains raises concerns about potential biases. Wastewater treatment plants serve as strategic sites for surveillance, with influent being a resource for monitoring regional AMR and establishing baseline data for downstream monitoring.

Aim: To investigate potential biases in ESBL-related genomic surveillance.

Method: Using influent, a whole genome sequencing comparative analysis was performed on 80 E. coli isolates grown on ESBL-selective plates and 201 E. coli isolates grown on nutrient agar with no antibiotics. Results: Significant differences in terms of AMR were observed between the two cohorts. The ESBL-selected isolates carried an average of six ARGs with the three most common being mphA (macrolide) > blaCTX-M-15 (ESBL) > tetA (tetracycline). Notably, 34 blaCTX-M genes were located on putative IncF plasmids and a near equal portion (n=33) were located chromosomally. Conversely, 80% of non-selected isolates carried no ARGs and no blaCTX-M genes were identified. Nevertheless, other critically important ARGs were only identified in the non-selected isolates, including tet(X4), known to inactivate all tetracyclines including “last resort” tigecycline and eravacycline. The ESBL-selected cohort was less diverse (23 sequence types [STs] vs 85) and heavily skewed towards ST131 (40% vs 2%). Several important pandemic pathogenic STs were only found in the non-selected cohort including ST12, ST58, ST73, ST95, ST117 and ST127. A genomic region (transmissible locus of stress resistance [tLSR]) conferring resistance to extreme heat, chlorination, and advanced oxidants was also only found in non-selected isolates (20%), and frequently (14%) on plasmids. Implications: the study advocates nuanced AMR surveillance, urging non-selected strain inclusion and consideration of broader resistance mechanisms, like tLSR to ensure resistance to other stressors is not missed.

Author: Veronica Jarocki, UTS, Australia

Co-Authors: Bogema, Daniel; Li, Dmitriy; Yam, Jerald; Jenkins, Cheryl; Hai, Faisal; Djordjevic, Steven

Session 2
Abstract 213
Room 3-4

Identification of metabolic and temporal niches and their association to the persistence of antibiotic and biocide resistant bacteria in wastewater.

Background/Aim: Antimicrobial resistant bacteria (ARB) can persist in the environment for long periods of time, despite metabolic fitness costs that arise with resistance. We investigate the role of differences in metabolic niches as a mechanism that allows ARB to persist in the environment. Specifically, our aim is to identify metabolic niches that can select for and against ARB.

Procedure/Method: 62 E. coli strains isolated from different WWTPs were used. The isolates have different levels of resistance to 14 antibiotics. The susceptibility of the isolates to the disinfectants didecyldimonium chloride, methylisothiazolinone and chlorhexidine was determined. In addition, genome scale metabolic models (GEMs) were constructed and the growth of the strains was simulated in the presence of 298 different carbon sources (C-sources). Furthermore, the growth rates of the isolates were measured for 3 different C-sources.

Findings/Results: Many of the isolates have high resistance to multiple antibiotics and a wide range of minimum inhibitory concentrations (MIC) (0.0015-32×clinical-breakpoint) but a very narrow MIC-range to the disinfectants (highest MIC up to 16×higher than the lowest MIC). GEMs identified 40 C-sources that supported growth in only a subset of the isolates. The accuracy of the GEMs in predicting growth on D-malate was 93%. A subset of 10 isolates was identified of which 5 isolates are resistant to ciprofloxacin, gentamicin and tetracycline and grow on sucrose but not on D-malate and the other 5 isolates are susceptible to the same antibiotics but grow on D-malate and not on sucrose.

Implications/Applications: Our data suggest that C-source availability could affect selection between resistant and susceptible bacterial strains. Currently, we use synthetic microbial communities to experimentally show that different metabolic environments can select for and against resistance. In the future, this concept could be applied to modulate natural microbial communities to reduce the number of ARB in environments like wastewater.

Author: Orestis Kanaris, BAM (German federal institute for material research), Germany

Co-Authors: Kreft, Jan-Ulrich; Schulz, Anne-Christine; Nordholt, Niclas; Schreiber, Frank

Monday, May 27 - 8:30-10:15

Back to the Program

Session 2

Abstract 399

Room 3-4

Clustering in Context: Antimicrobial Resistance and MGE Dynamics in ESKAPE Pathogens Circulating across Environmental and Used WWTP Waters in Romania

Background: Romania faces high rates of multidrug-resistant pathogens, particularly the ESKAPE group, increasingly resistant to last-resort antimicrobials. WGS monitors transmission, tracks high-risk pathogens and investigates resistance factors in environmental waters. Understanding these mechanisms is crucial for implementing antimicrobial resistance programs and public health initiatives.

Methods: This five-year study (2017-2022), collected & sequenced ~900 ESKAPE bacterial strains (K.pneumoniae (K.p.), A.baumannii (A.b.), E.coli (E.co.) – most abundant ones) from environmental surface waters, WWTPs and clinical settings in major Romanian cities. Approximatively 200 surface- & 300 used-waters strains have been further analysed with the use of various bioinformatic setups and in-house scripts.

Results: The preliminary results show increased repertoires of antimicrobial factors. Biocide resistance (BR) is observed in all bacterial species, some containing full efflux systems (e.g. ade systems found in A.b.), and E.co. exhibiting the highest abundance of BRGs (efflux systems and metal resistance mainly). A.b. strains also show a notable presence of qacEdelta1. Antibiotic resistance genes (ARGs) are most abundant in A.b., E.f. and K.p. species. Specific genes like vanA/SA/XA/YA/ZA in E.f. and blaADC-25, blaOXA-23, blaOXA-66 in A.b. are consistently found. MGEs are prevalent, with ICEs abundant in K.p., IS in A.b., E.co. and K.p. (also high in upstream waters), and high Tn abundance in A.b. and K.p.

Context analysis, conducted through in-house scripts, reveals gene clusters allowing a comprehensive exploration of relationships between various ARGs, BRGs, VFs and MGEs elements. While most contigs contain few elements, some form clusters with up to 80. Notably, an increase in VF numbers (n>=20) indicates the presence of pathogenicity islands (PIs), often associated with BRGs, ICEs and phages. Species-dependent preferences emerge, with K.p. favouring ICEs, while E.co. and A.b. show a preference for BRGs and phages. Despite similar numbers between surface/used water samples, substantial clusters are evident in downstream samples too.

Acknowledgements:

PN-III-P1-1.1-PD-2021-0540(PD102/2022),UEFISCDI;

PN-III-P1-1.1-TE-2021-1515(TE112/2022),UEFISCDI.

Author: Marius Surleac, National Institute for Infectious Diseases “Prof. Dr. Matei Bals”, Romania

Co-Authors: Czobor Barbu, Ilda; Paraschiv, Simona; Gheorghe-Barbu, Irina; Rotaru, Liviu-Iulian; Otelea, Dan; Chifiriuc, Carmen

Monday, May 27 - 11:00-12:30

Back to the Program

Session 3
Abstract 216
Room 4

Bioaerosols and Antibiotic Resistance Gene Transport: Understanding the Role, Impacts, and Fate

Background and Aim: A vast project, titled “Antimicrobial resistance genes (ARG) in bioaerosols in Canadian arctic, rural and urban environments: sources, profiles, transport, and fate” was funded by the 2019 Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Frontiers program. This project involving scientists from broad ranges of expertise (bioinformatics, bioaerosols, veterinary medicine, engineering, medical doctors, epidemiologists, modeling experts, and physiologists) has led to broad air sampling campaigns, providing rich information on various bioaerosol sources for antibiotic resistance genes both in natural and man-made environments.

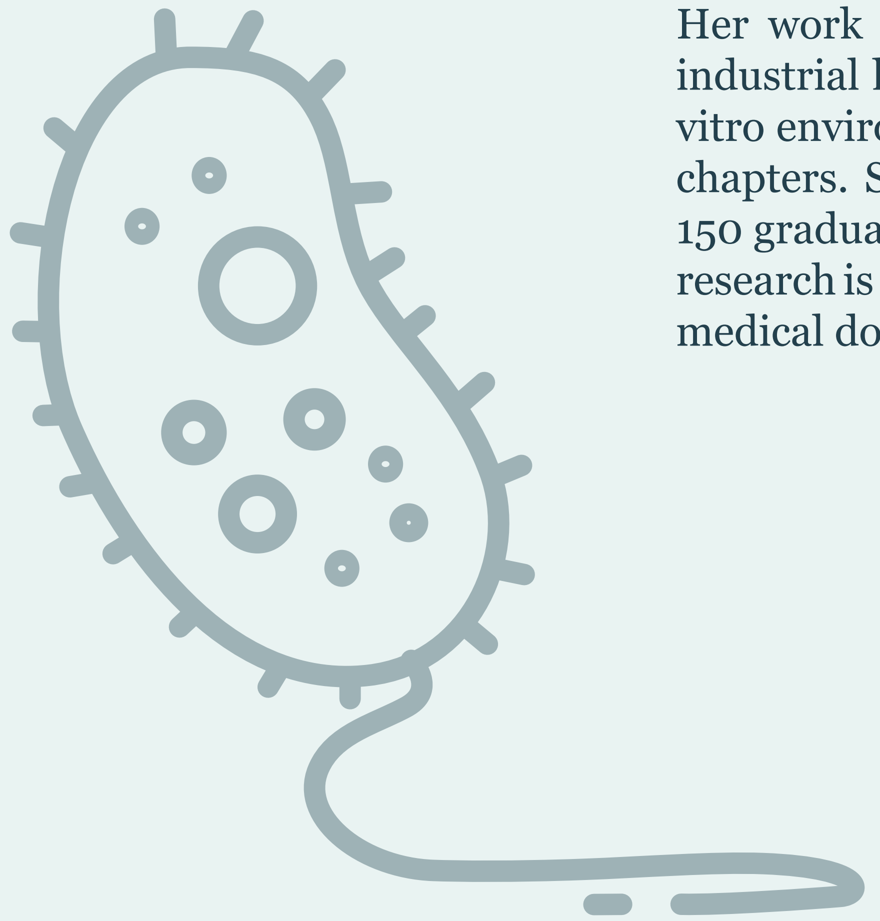
Method: We have captured air samples from clouds, atmospheric particles, trans-Atlantic and Northern environments, hospitals, agricultural (pig, poultry, and fish) farming and manure spreading activities, and wastewater treatment. The project also explores alternative air sampling methods such as conifer needles, HVAC systems filters, and car air cabin filters that were used for a trans-Canada survey of circulating ARGs. A high-throughput PCR assay was also developed to increase analytical capacity. Findings. Our data will be used for dispersion modeling, risk assessment models, and in vivo ARG transfer. Data curation was secured through an extensive database and sample preservation system to ensure the sharing of information and material for future collaborations.

Implications: This project is the first initiative to cover broad sources of bioaerosols and integrate the findings into models to better understand the role of air in the dispersion of ARG. The presentation will cover the methods, context and possibility of collaborations for future valorization of samples and data.

Author: Caroline Duchaine, Université Laval, Canada

Biography:

Caroline Duchaine is a full professor in the Department de biochemistry, microbiology et bioinformatics at Université Laval and holds the Canada Research Chair (Tier 1) in Bioaerosols. Her work focuses on the study of bioaerosols in a variety of contexts: human, animal and industrial health, as well as on the development of analytical strategies for both natural and in vitro environments. She has co-authored over 660 abstracts, research papers, reports and book chapters. She has conducted 135 funded research projects and participated in the training of 150 graduate students. She has won several awards, both for her research and her teaching. Her research is transdisciplinary and involves collaboration with various experts, including physicists, medical doctors, veterinarians, engineers and bioinformaticians.



Session 3
Abstract 212
Room 4

Antimicrobial resistance dissemination potential of bio-based fertilizers according to field trials

Background and Aim: Use of bio-based fertilizers (BBFs) could offer solutions for reducing our dependence on fossil energy and for tightening the nutrient cycles. BBFs are a heterogeneous group of recycled fertilizers including products manufactured from manures, sewage sludges, composts, ash, biowastes, slaughterhouse and crop residues as well as mixtures of all these. Since BBFs are new types of materials, their usability and risks should be determined.

Methods: The LEX4BIO-project (https://lex4bio.eu/) aims to produce a science-based toolkit for the use of BBFs in agriculture. As a part of LEX4BIO, we analyzed the antimicrobial resistance (AMR) potential of metagenomes from 528 soil samples. The samples were obtained from field trials in Finland, France and Spain conducted for analyzing Phosphorus and Nitrogen efficiency as well as risks caused by heavy metals and organic contaminants. Samples were taken before the BBF application, immediately after the application, and six weeks after the application.

Findings: The results show that BBFs in general have low AMR dissemination potential. Poultry production side stream BBFs were an exception, possibly due to high concentrations of antibiotic residues in these BBFs. The abundances and diversities of AMR genes were elevated in soils after application of BBFs manufactured from materials such as manures, composts, or sludges, but returned close to those of soil samples taken before BBF application within six weeks. The BBFs had no negative effect on the microbiome diversity or beneficial microbes such as mycorrhiza. The analyses will continue by exploring connections between AMR and chemical data from the soils.

Implications: Given the large number of samples and carefully designed experiments, the results could give valuable understanding on the ecology of AMR. In our presentation, we will further elaborate and discuss the current and developing results.

Author: Marko Virta, University of Helsinki, Finland

Co-Authors: Muurinen, Johanna; Markkanen, Melina; Hultman, Jenni; Ylivainio, Kari; Jansen, Boris; Salo, Tapio; Delgado, Antonio; Recena, Ramiro; Arkoun, Mustapha; D’Oria, Aurelien; Keskinen, Riikka;

Monday, May 27 - 11:00-12:30

Back to the Program

Session 3

Abstract 260

Room 4

Replacing untreated with treated wastewater for irrigation: Consequences for antibiotics, metals and nutrients in soils

Background and Aim: Re-use of wastewater for irrigation in agriculture is widespread and will increase to reduce water scarcity caused by population growth and climate change. To reach the Sustainable Development Goal “Clean Water and Sanitation”, investments in wastewater treatment have been and are being made. We hypothesize that replacing untreated wastewater with treated wastewater in long-established wastewater irrigation systems can cause the mobilization of pollutants like heavy metals and antibiotics that accumulated in soils in the past, e.g., due to increased mineralization of soil organic matter as a consequence of reduced nutrient and organic matter inputs with irrigation water.

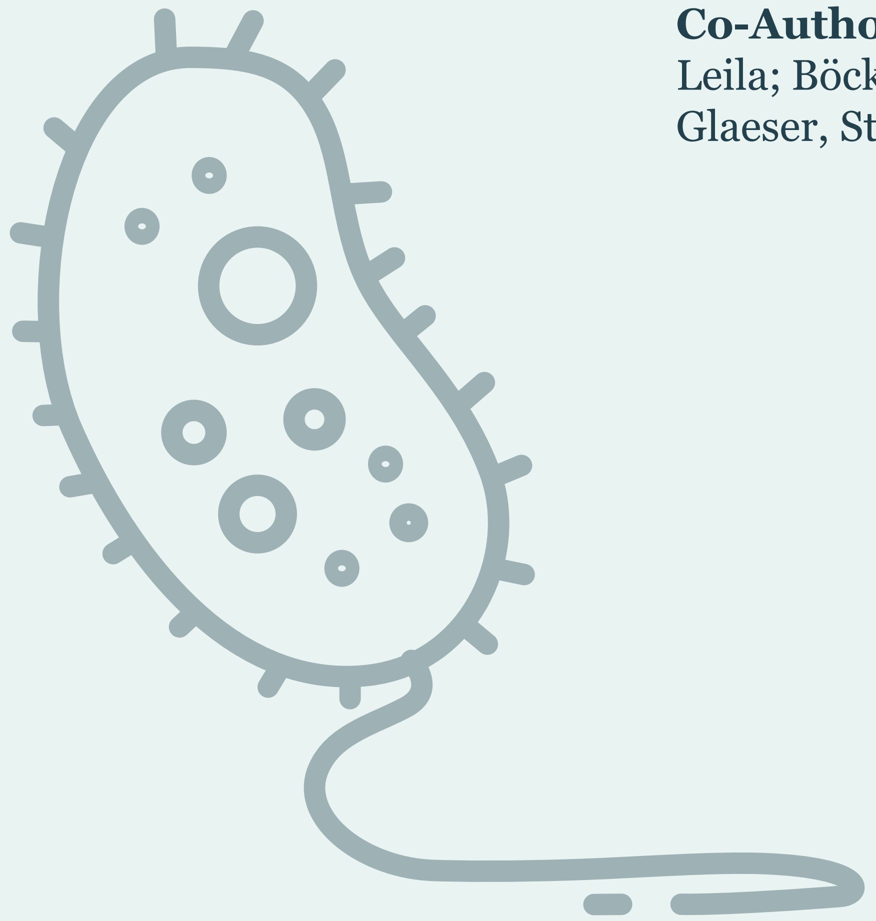
Method: We incubated samples of 3 different soil types from the Mezquital Valley, Mexico, which have been irrigated with untreated wastewater for > 80 years, with either a) untreated Mexico City wastewater, b) effluent of the Atotonilco wastewater treatment plant, c) untreated wastewater spiked with antibiotics and disinfectants, and d) effluent spiked with antibiotics and disinfectants, all for a period of eight weeks. Extractable soil concentrations of metals, antibiotics, organic carbon, nitrogen and phosphorus were determined for eight points of time during the incubation.

Results: Although the effluent contained less organic carbon, nitrogen and phosphorus than untreated wastewater, only water-soluble nitrogen concentrations of soils were reduced in soils incubated with effluent instead of untreated wastewater. Water treatment did not reduce water-soluble concentrations of antibiotics in soils. However, dissipation of sulfamethoxazole in soils mixed with spiked effluent appeared retarded compared to soils mixed with spiked untreated wastewater. The most clayey soils had higher water-soluble soil concentrations of zinc and copper when incubated with effluent compared to soils incubated with untreated wastewater.

Implications: The implementation of water treatment on long-established wastewater irrigation systems might open a time-window of risk during which mobilized metals and persistent antibiotics select antibiotic resistant bacteria in soils.

Author: Jan Siemens, Justus Liebig University Giessen, Germany

Co-Authors: Heyde, Benjamin; Lüneberg, Kathia; Hahn, Nicole; Axtmann, Katharina; Soufi, Leila; Böckmann, Matthias; Braun, Melanie; Amelung, Wulf; Bierbaum, Gabriele; Gallego, Sara; Glaeser, Stefanie; Grohmann, Elisabeth; Smalla, Kornelia; Zarfl, Christiane; Siebe, Christina



Session 3

Abstract 40

Room 4

Trade-offs of increasing temperatures for the spread of antimicrobial resistance in river biofilms

River microbial communities regularly act as the first defence against antimicrobial resistance genes (ARGs) that enter environmental microbiomes through wastewater. The global increase in temperatures due to climate change might disrupt this barrier effect by altering river microbial community structure and functions as well as resistome composition. Furthermore, the invasion dynamics of wastewater-born ARGs into river biofilm communities could be affected.

To combat the spread of antimicrobial resistance (AMR) in the environment under changing global temperatures, it is hence imperative to study such temperature effects on the natural river microbial resistome as well as the river microbial communities’ resistance to invasion of foreign ARGs. Here we aimed at elucidating such temperature effects on the naturally occurring river biofilm resistome, as well as invasion success of foreign ARGs entering through wastewater.

To achieve this, natural river biofilms were grown in pristine rivers and transferred to laboratory recirculation flume systems operated at three temperatures (20°C, 25°C, 30°C). Already after one week of temperature acclimatisation, significant increases in the abundance of naturally occurring ARGs were detected at higher temperatures. After this acclimatisation period, biofilms were exposed to a single pulse of wastewater, and invasion dynamics of wastewater-born ARGs were analysed over two weeks. Initially, after one day, wastewater-born ARGs were throughout able to successfully invade all biofilms with no observable temperature effect on their relative abundance. However, thereafter ARGs were lost at a far increased rate at 30°C, with ARG levels dropping to the initial natural levels after 14 days. Contrary at the lower temperatures, ARGs were either lost at slower rates or even able to establish themselves in biofilms with stable relative abundances above natural levels. Hence, higher temperatures come with trade-offs for river resistomes: Naturally occurring ARGs increase in abundance, while foreign, invading ARGs are lost at elevated speed.

Author: Thomas U. Berendonk, Technicak University Dresden/Indian Institute of Technology Roorkee, India

Co-Authors: Klümper, Uli; Kneis, David; Padfield, Daniel; Szekeres, Edina; Teban-Man, Adela; Coman, Cristian; Singh, Gargi; Bagra, Kenyum

Monday, May 27 - 11:00-12:30

Back to the Program

Session 4
Abstract 429
Room 3

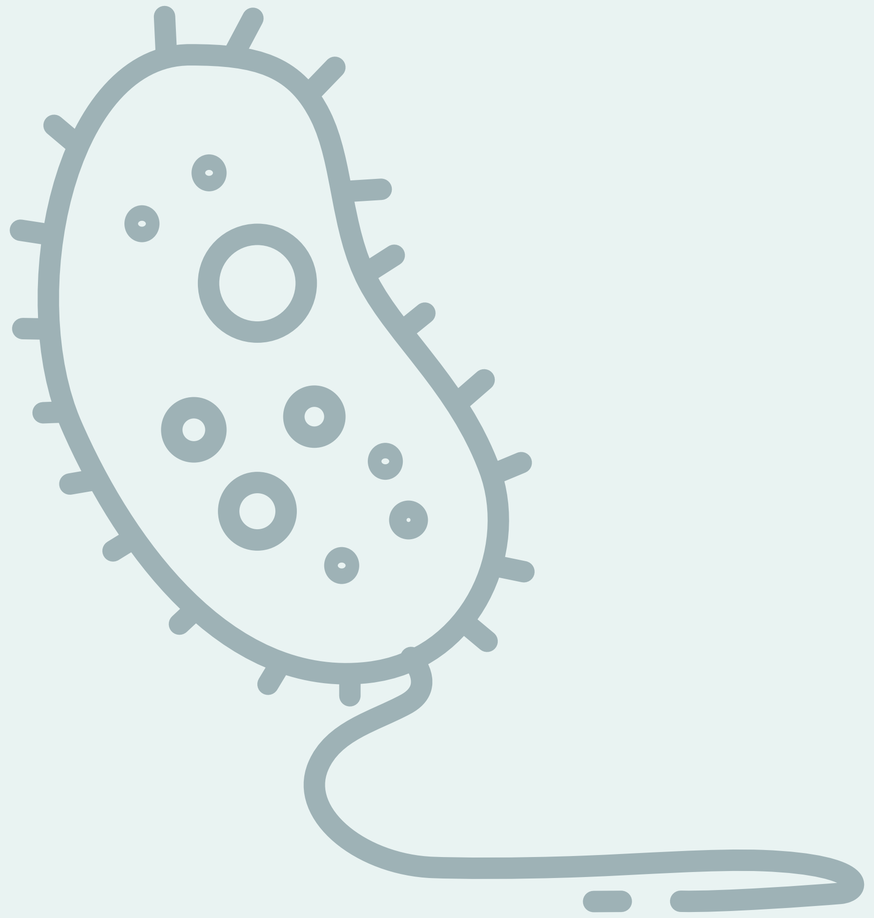
Antibiotic resistance genes (ARGs) as environmental contaminants: Where are we now?

Nearly two decades ago, the first scientific research studies were published that collectively began to make the case that antibiotic resistance genes (ARGs) can be considered as environmental contaminants. In sum, ARGs can enable pathogens to resist antibiotic treatments and thus are undesirable from a human health standpoint, while at the same time numerous examples began to reveal how anthropogenic activities can contribute to their spread via environmental pathways. Since this time, understanding of ARGs as environmental contaminants has continued to grow and evolve, but this understanding has been shaped by the evolution of methods as the lens through which we detect, quantify, and track their movement in various environments. Here we will examine how various methods, including PCR-, culture-, and DNA-sequencing-based methods, continue to shine light on our understanding of ARGs as environmental contaminants. Finally, we will consider policy implications of ARGs as contaminants, including measures that can be taken in terms of local and global coordination of monitoring and mitigation strategies.

Author: Amy Pruden, Virginia Tech, United States

Biography:

Prof. Amy Pruden is the W. Thomas Rice Professor and University Distinguished Professor in the Department of Civil and Environmental Engineering at Virginia Tech. She earned her B.S. in Biological Sciences (1997) and her PhD (2002) in Environmental Science, both at the University of Cincinnati. Her research is focused on understanding the fate of antibiotic resistance genes (ARGs) in aquatic, terrestrial, and airborne environmental systems. She is currently leading a transdisciplinary PhD training grant funded by the US National Science Foundation focused on translating metagenomic-based surveillance of AMR into policy and practice. Pruden is the recipient of the Presidential Early Career Award in Science and Engineering, the Paul L. Busch Award for innovation in water research, the Recipharm International Environmental Award, and is a fellow of the International Water Association.



Session 4
Abstract 373
Room 3

Cultivation strategies have a strong impact on studies of antimicrobial resistant bacteria in the environment

Cultivation of antimicrobial resistant bacteria (ARB) is more and more recognized as a key strategy to understand the spread of antimicrobial resistance (AMR) in the environment. For many years, extraction of environmental DNA and quantification of antimicrobial resistance genes (ARG) was used by many research groups to target environmental AMR spread. But, especially the multi-resistance status of bacteria or plasmid content cannot by covered by these methods and new resistance strategies are overseen. These important data can be obtained by using specific cultivation approaches. However, the cultivation strategies must be carefully selected and critically evaluated to get reliable results.

By selecting specific media and incubation conditions, ESKAPE bacteria can be selectively cultured. By supplementing antibiotics to these media, subgroups of resistant bacteria can be cultured. Nevertheless, there are several challenges which can strongly impact the outcome of these cultivation efforts. Growth of more abundant intrinsically resistant non-target bacteria can disturb the detection of ARGs or low abundant or viable but non culturable (VBNC) potential pathogenic ARBs in environmental samples. This may affect their direct cultivability on agar media. Non-selective or selective pre-enrichment strategies enhancing the cultivability of specific bacteria but can also influence the outcome of these studies, because some strains may have growth benefits during pre-enrichment. Resistance gene exchange during pre-enrichment may also occur.

We will give a critical overview of cultivation efforts for the research on AMR based on our knowledge we obtained in the last 15 years in our AMR research collaborative network projects (the BMBF funded projects RiskAGuA and the JPI AMR project ARMIS in which the spread of AMR from manure and biogas plant digestates were studied, and the DFG research group PARES in which the spread of AMR in Mexican soils after a shift from irrigation with untreated to treated wastewater is monitored.

Author: Stefanie P Glaeser, Justus-Liebig University Giessen, Germany

Co-Authors: Kämpfer, Peter; Pulami, Dipen; Balchandran, Sanjana; Chifiriuc, Carmen; Schmitt, Heike; Scott, Andrew; Topp, Ed; Chalmers, Gabhan; Ricker, Nicole

Monday, May 27 - 11:00-12:30

Back to the Program

Session 4

Abstract 120

Room 3

Bathing Water Based Epidemiology – five years AMR surveillance at Scottish bathing water sites (2018-2023)

Background and Aim. Wastewater-based epidemiology (WBE) provides data on the prevalence and spatiotemporal spread of AMR in hospitals and the wider community. Bathing water-based epidemiology (BBE) extends this to include a wider catchment area reflecting AMR from all one health sectors and has the potential to assess risk of transmission to humans. Here we present data from five years of AMR surveillance at 86 designated bathing water (BW) site in Scotland.
Procedure/Method. During the Scottish bathing water season (June to September; 2018-2023) water samples were collected (on up to 18 occasions) from each of 86 BW sites - freshwater (3) and coastal water (83). Total E. coli and cefotaxime resistant (CTX-R) E. coli were enumerated by membrane filtration on TBX media with and without 4mg/L cefotaxime.
Findings/Results. A total of 6,946 samples were tested (average of 1,389 per year). E. coli were detected in 4701 samples (68%). Over the five years CTX-R E. coli were detected in 10% (668) of samples - intra year variation ranged from 6% to 19%. The majority (88%) of CTX-R positive samples contained ≤10 CFU/100ml. Each year CTX-R E. coli were detected on average at 51 (range 43-67) BW sites. The proportion of E. coli that were CTX-R resistance was 0.4% (range 0.3% to 0.8%). Multidrug resistant isolates were detected at 12 BW sites.
Implications/Applications. The proportion of CTX-R E. coli (0.4%) in Scottish BW is markedly lower than in Scottish human clinical isolates (8%) but is similar to the proportion in raw (0.4%) and treated (0.6%) sewage from a large Scottish WWTP. BW is an exposure relevant site where users are estimated to ingest up to 2.8 CTX-R E. coli per session. These Scottish data are the only EU example of bathing water AMR surveillance being included in national scale monitoring.

Author: Donald Morrison, Edinburgh Napier University, United Kingdom

Co-Authors: Findlay, David; O’Keefe, Brian; Blaud, Aimeric; Redshaw, John

---

Session 4

Abstract 241

Room 3

Genomic and metagenomic insights into antimicrobial resistance in human, poultry, and wastewater environments in urban and rural Bangladesh

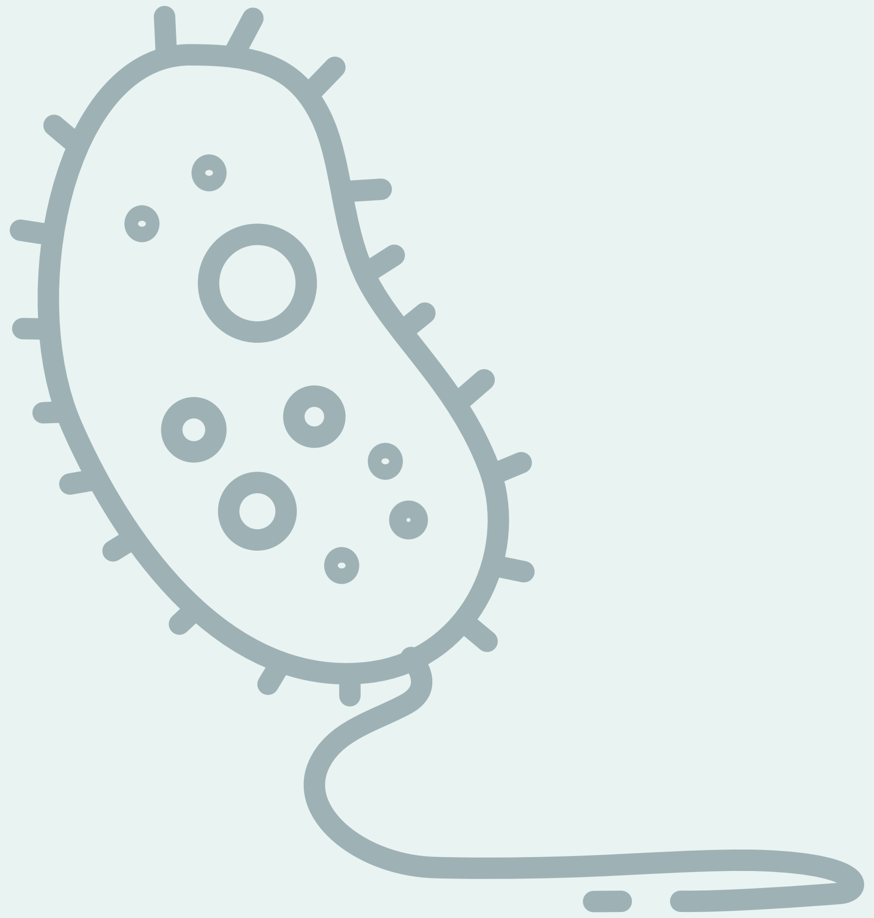
Background and aim: Antimicrobial resistance (AMR) poses a significant threat to global health and sustainable development goals, especially in low- and middle-income countries (LMICs). Interactions between humans, animals and the environment are considered critical foci for addressing AMR. However, One Health data on AMR in LMICs are presently scarce. In this study we aimed to address this knowledge gap.

Method: We analyzed whole genome sequences (WGS) of 117 extended-spectrum β-lactamase-producing Escherichia coli (ESBL-Ec) isolates obtained from human (n=20), poultry caecal (n=12), and wastewater (n=85) of rural households (n=48), rural poultry farms (n=20), and urban wet markets (n=49) in Bangladesh. In addition, we investigated fecal antibiotic resistomes in humans with and without intensive poultry exposure in urban wet markets (n=13) and rural households (n=7) along with poultry caecal (n=10) and wastewater (n=10) from the same setting using shotgun metagenomic analysis.

Findings: No significant differences in antibiotic resistance genes (ARGs) were observed in ESBL-Ec isolated from humans with and without poultry exposure. Likewise, poultry exposure did not significantly alter the human faecal resistome. Wastewater had the highest ARG richness, though this was partially explained by poultry caecal and human faecal sources. Similarly, wastewater isolates showed higher ARG diversity than human and poultry isolates. No clonal transmission between poultry and human isolates was found, but wastewater was a reservoir for ESBL-Ec for both. Putative sharing of ARG-plasmids was observed between human/poultry and wastewater isolates. However, bacterial isolates sharing plasmids were also clonally related, suggesting clonal spread was more probable than just plasmid transfer.

Implications: Our study underscores the critical role of wastewater in the propagation of antibiotic-resistant bacteria and antibiotic resistomes. Wastewater is an important, but poorly understood component of One Health studies on AMR in Bangladesh. Future investigations should prioritize efforts towards gaining a deeper understanding of this crucial component.

Author: MOHAMMAD ISLAM, Washington State University, United States



Back to the Program

Session 5
Abstract 426
Room 4

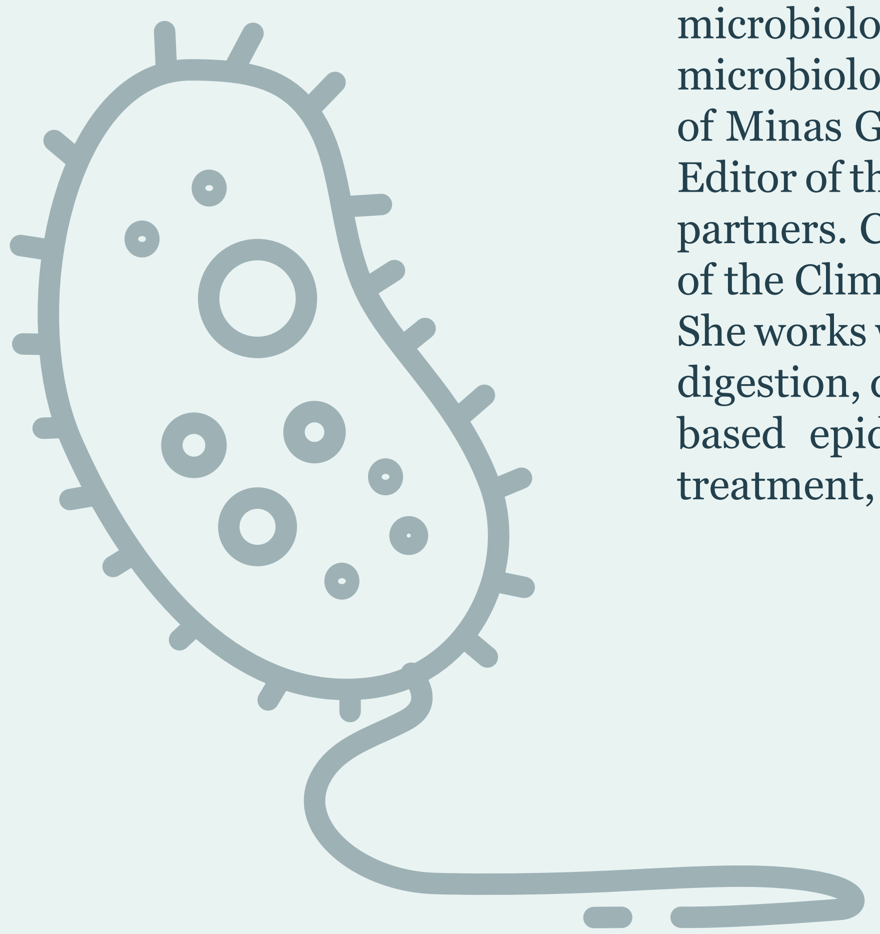
Sewage Surveillance of Antibiotic Resistance in Brazil as revealed by long-term monitoring of wastewater treatment plants

Wastewater treatment plants (WWTP) are important sources of Antibiotic Resistant Bacteria (ARBs) and genes (ARGs), and play an important role in removal and dissemination of antibiotic resistance (AR) in the environment. Detailed information about AR removal by the different biological treatment technologies (aerobic and anaerobic) is needed, particularly in Brazil. This study investigated the occurrence, removal and identification of ARBs and ARGs in four full-scale WWTPs: WWTP-A (conventional activated sludge-CAS), WWTP-B (UASB reactor followed by biological trickling filter), WWTP-C (modified activated sludge followed by UV disinfection-MAS/UV) and WWTP-D (facultative and maturation ponds). Samples of raw sewage (RI) and treated effluent (TE) were collected and, through the cultivation-based method and qPCR, bacteria resistant to different antibiotics and genes were quantified over more than one-year period. MAS and ponds were effective in reducing ARB counts (2 to 3 log removal), compared to CAS (1 log removal) and UASB/BTF (0.5 log). ARGs removal varied (0.55 to 2.0 logs), depending on the gene: intI1, sul1 and TetA was about 0.2 to 0.5 log removal. blaTEM, ermB and qnrB removals were from 1 to 2 logs by CAS, UASB/BTF and MAS/UV. In general, the ponds in series showed better performance (3 logs removal) likely due to the high hydraulic retention time. ARB and ARG concentrations in treated effluents were higher than those reported in the literature from other countries using the same treatment technologies. Understanding the specific constraints and capabilities of each treatment technology is critical to advancing and improving these facilities to reduce the spread of AR in the environment.

Author: Juliana Calábria de Araújo, Universidade Federal de Minas Gerais, Brazil

Biography:

Juliana graduated in Biological Sciences at the Federal University of Rio de Janeiro in 1992. PhD in Sanitary Engineering at the University of São Paulo (2001), with an internship period at EAWAG (Switzerland). Postdoctoral in Environmental Microbiology at USP, and in Anaerobic microbiology at University of Toronto (2019). She is an Associate Professor in wastewater microbiology at the Department of Sanitary and Environmental Engineering at Federal University of Minas Gerais, Belo Horizonte, Brazil. Co-author of more than 80 scientific works. Associate-Editor of the Cleaner Water journal. She coordinated several national projects/with international partners. Currently, she coordinates the Genomic Sewage Surveillance Project and is a member of the Climade (Climate amplified Diseases & Epidemics) Consortium.
She works with environmental and applied microbiology, microbial molecular ecology, anaerobic digestion, detection of pathogens, antimicrobial resistance in water and wastewaters, wastewater based epidemiology, Anammox-based process for nitrogen removal, biological wastewater treatment, among other topics.



Session 5
Abstract 48
Room 4

Diversity and dynamics of antimicrobial resistome in activated sludge across continents

Background and Aim: Activated sludge is a known reservoir of antibiotic resistance genes (ARGs) that can potentially contaminate environmental waters through wastewater treatment effluent. However, the geographic distribution of ARGs within activated sludge remains poorly understood, despite the global diversity of wastewater. This study aims to elucidate the diversity and dynamics of ARGs within activated sludge from wastewater treatment plants (WWTPs) across different continents.
Methodology: We collected shotgun metagenomic sequence data from activated sludge and influent wastewater samples (n = 62) at full-scale WWTPs in the USA, Europe, Japan, and China between 2015 and 2019, obtained from INSDC databases. The ARG composition of each sample was determined by aggregating and normalizing reads of each ARG obtained by blasting with CARD v.3.2.6.
Results: Our findings reveal both common features and continental disparities in the antimicrobial resistome within activated sludge. Total ARG abundance in activated sludge consistently appeared lower and less variable than in influent wastewater across all countries. The ARG compositions in activated sludge exhibited reduced regional diversity to those found in wastewater. Many of the ARGs abundant in activated sludge were dependent on the prevalence of ARGs in influent wastewater from each respective country. Notably, sul1, AAC(6')-Ib7, oleC, and blaOXA were detected at higher abundance in activated sludges across countries than in wastewater, suggesting the persistence of these ARGs within wastewater treatment systems. Conversely, mph, msr, and tet gene families tended to be eliminated in activated sludge samples from all countries. Among mobile genetic elements, intI1, and insertion sequences like istA, istB, and IS91 were more abundantly detected in activated sludge compared to wastewater.
Implications: Regarding commonality of antimicrobial resistome found in activated sludge across different countries, specific ARGs may serve as indicators for a globally integrated monitoring framework for antimicrobial resistance, aiding in strategies to mitigate its environmental dissemination.

Author: Muhammad Adnan Sabar, Kanazawa University, Japan

Co-Authors: Morinaga, Yuta; Matsuura, Norihisa; Watanabe, Toru; Honda, Ryo

Back to the Program

Session 5

Abstract 301

Room 4

Complex Fate of Antimicrobial Resistance Genes in Activated Sludge Determined by Solids Retention Time

Background/Aim: Microbial immigration, or community coalescence, between the influent and activated sludge has emerged as a key factor impacting the abundance and diversity of antimicrobial resistance genes (ARGs) in wastewater treatment plants (WWTPs). However, a comprehensive understanding of factors impacting immigration dynamics and the movement of ARGs remains elusive. Using a series of highly controlled reactors, the current study aimed to investigate the impact of operational solids retention time (SRT) on immigration and the fate of antimicrobial resistance (AMR) in activated sludge WWTPs.

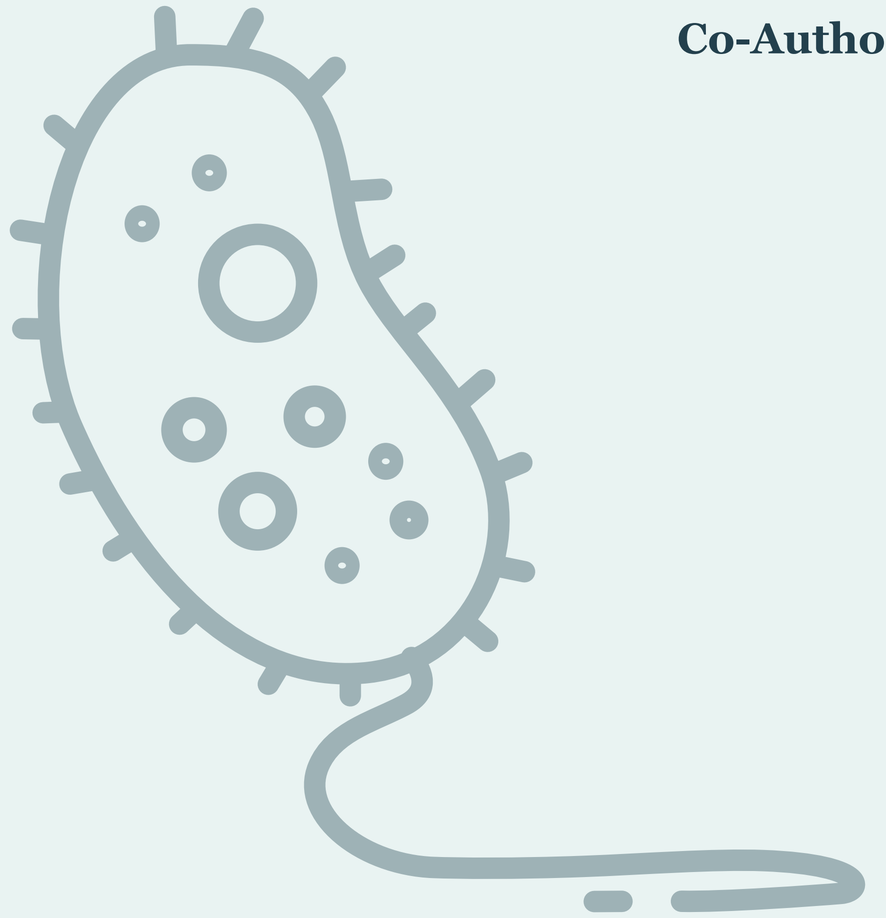
Method: A series of triplicate reactors were operated at five different SRTs ranging from 1.8 to 14 days, receiving a synthetic wastewater feed supplemented with influent solids harvested from a full scale WWTP. 16S rRNA gene sequencing determined the compositions of the microbial communities, and high-throughput qPCR array (Resistomap) quantified ARG abundances in each reactor set.

Results: SRT played a critical role in the formation of unique microbial communities in the reactors. Contradicting previously published studies, immigration had a greater impact on the microbial community at higher SRTs. The ARG dynamics at the immigration interface were observed to be complex. While the abundance of genes such as blaGES and ermB significantly increased with immigration, others such as blaVIM and vanB were unaffected. Furthermore, the abundance of genes including vanA and cfxA positively correlated with SRT, while mobile genetic elements such as IntI2 were negatively correlated. By further defining the reactor microbial communities into specific populations, the observed ARGs dynamics could be related to the microbial community assembly process for the first time.

Implications/Application: The results from this study demonstrate that the fate of ARGs in WWTPs is highly variable and complex. In the future, studies aiming to reduce AMR in WWTPs should carefully consider which ARGs to prioritize, and the factors impacting their persistence within this environment.

Author: Claire Gibson, McGill University, Canada

Co-Author: Frigon, Dominic



Session 5

Abstract 217

Room 4

Exploring AMR in conventional and advanced wastewater treatment processes: A cross study along the oxygen gradient

Background and Aim: Antimicrobial resistance (AMR) is a global concern and wastewater treatment plants (WWTPs) are scrutinized for impacting AMR determinants, including antibiotic-resistance genes (ARGs) and mobile genetic elements (MGEs). WWTPs strive for minimisation of energy consumption, introducing new technologies like aerobic granular sludge (AGS), membrane aerated biofilm reactors (MABR) and anaerobic ammonium oxidation (Anammox) as alternative for conventional activated sludge (CAS) systems. Processes and conditions influence microbial communities and AMR. This cross- process study aims at examining one major factor, oxygen, differentiating the processes into aerobic and anaerobic classes.

Procedure/Method: We conducted a comparative analysis of AMR, including biological nutrient removal (CAS and AGS), partial nitration-anammox (PN/A), and anaerobic digestion (AD) processes. We combined metagenomics and qPCR to analyse the compositions of the microbiome, resistome, and mobilome.

Findings/Results: In our latest study from CAS, AD and PN/A stages combined in the same treatment line, metagenomics revealed that ARG prevalence in PN/A (0.26 ARGs/16S-rRNA-gene) surpassed all the preceding stages by up to 50%. AD demonstrated the lowest abundance (0.04 ARGs/ 16S-rRNA-gene) opposing one hypothesis of low/no oxygen conditions driving AMR increase. Previously, we reported that oxygen conditions influenced the abundance of ARGs and MGEs where AD operated with microaeration at low oxygen dosage (0-10 mg O2 g-1 CODfeed) exhibited at least 2.3 times higher prevalence of ARGs and MGEs compared to a higher oxygen dosage (50-150 mg O2 g-1 CODfeed), measured using qPCR. However, the same concept did not apply to different stages in the AGS non-aerated and aerated phases or CAS anoxic-aeration zones; no significant difference was obtained.

Implications/Applications: Recognizing AMR neglect in conventional WWTP designs, it is imperative to assess AMR profiles, dissemination, and risks for future designs and extensions of WWTPs; without compromising water quality. This study triggers more hypothesis tests and practical considerations on pollution control.

Author: Stella Christou, University of Surrey, United Kingdom

Co-Authors: Calderón-Franco, David; Nnorom, Mac-Anthony; Liu, Yang; van Loosdrecht, Mark; Guo, Bing; Weissbrodt, David; Ritchie, Jennifer

Monday, May 27 - 13:45-15:30

Back to the Program

Session 5
Abstract 132
Room 4

Changes to antimicrobial resistance (AMR) following waste water treatment processes: a UK-wide investigation

Background/Aim: Wastewater treatment works (WwTWs) play an important role in the release of AMR and AMR-driving chemicals into the environment. This study reports on part of the nationwide UK Water Industry Research Chemicals Investigation Programme 3, which aimed to better understand the role that WwTWs play in treating and transforming antimicrobial resistance (AMR) across different stages of treatment.

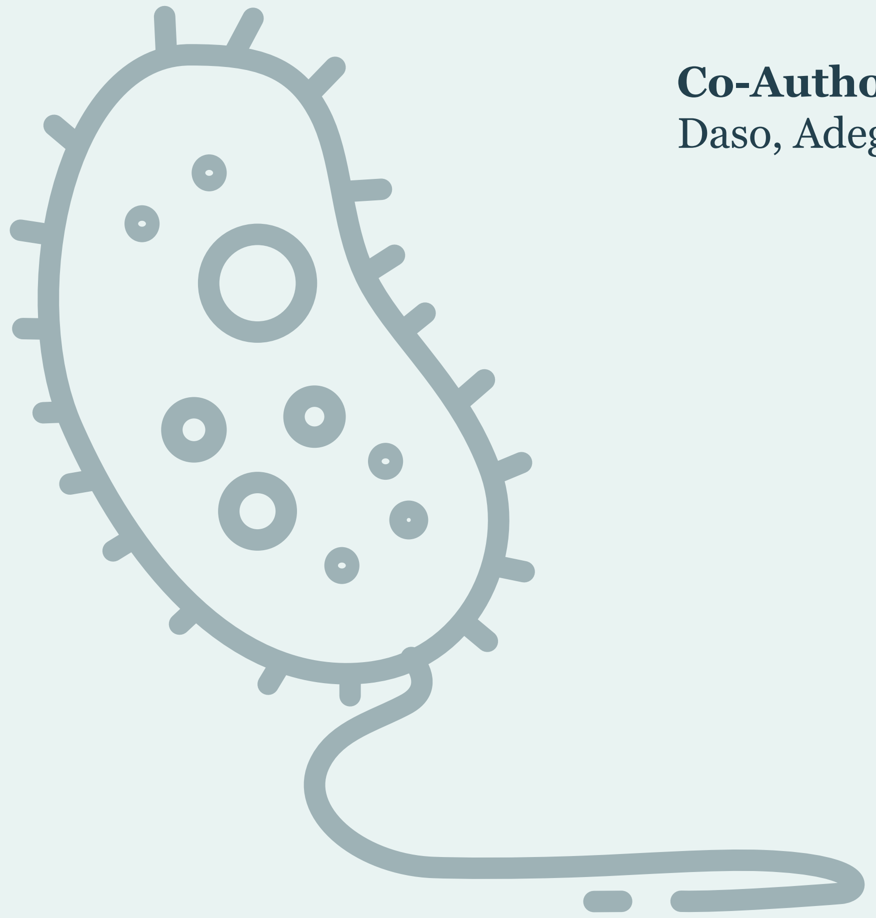
Method: Ten different WwTWs with mixed secondary and tertiary treatment processes were sampled on six occasions over 11 months in 2020 and 2021. 24-hour composite samples were taken from raw influent and final effluent and were analysed for AMR using qPCR and metagenomics. Contextual measurements included physicochemical parameters, dissolved metals, and 87 antibiotics/metabolites.

Results: Analysis using qPCR showed WwTWs were effective at reducing the abundance of antibiotic resistant genes (ARGs) in the final effluent (97.5% of influent/effluent pairs had negative log2fold reductions). For ARG prevalence, some reductions were seen between raw influent and final effluent (ermB and tetM), some increases (qacEdelta1) and a WwTW-dependent mix of increases and decreases (intI1, qnrS, and sul1). Paired analyses of metagenomic data highlighted that all WwTWs (regardless of treatment mechanism/configuration) had statistically significant declines in total ARG prevalence. However, analyses of differentially abundant genes in raw influent or final effluent found qacE, sul1, sul2, three ARGs from the ANT(3”) gene family, six OXA beta-lactamases and two macrolide esterases (ereA and ereD) were associated with final effluent, indicating potential selection for these ARGs or ARG-harbouring bacteria during wastewater treatment.

Implications: Examining AMR reductions throughout wastewater treatment is necessary to understand the nature and composition of AMR released into the environment. We show that against a background of ARG removal, a subset of ARGs increase in prevalence during wastewater treatment. Evaluating UK-wide changes in AMR following treatment aids the implementation of data-informed mitigation strategies to limit environmental pollution.

Author: Holly Tipper, UK Centre for Ecology and Hydrology, United Kingdom

Co-Authors: Newbold, Lindsay; Read, Daniel; Johnson, Andrew; Kasprzyk-Hordern, Barbara; Daso, Adegbenro Peter; Bowes, Mike; Gweon, H. Soon; Pereira, M. Glória; Singer, Andrew



Monday, May 27 - 13:45-15:30

Back to the Program

Session 6

Abstract 425

Room 3

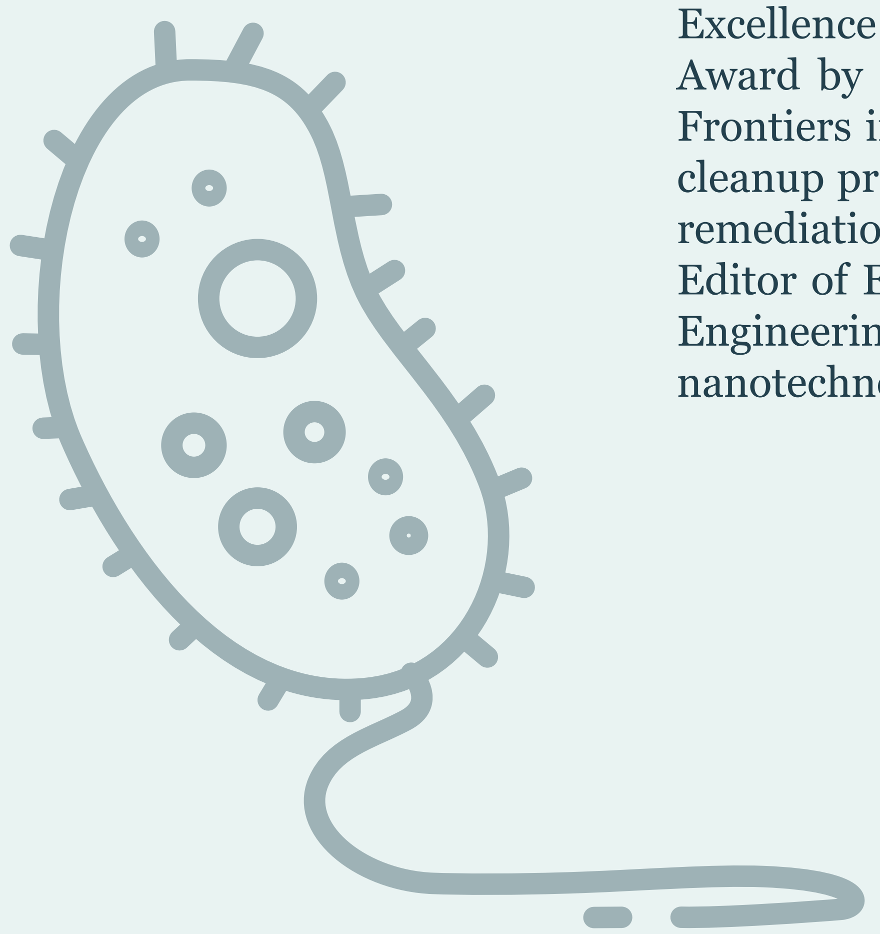
Proliferation and Phage-Based Control of Multidrug-Resistant "Superbugs" in Sewage Treatment Plants

Antibiotic-resistant bacteria (ARB) pose an imminent threat to global health. Their propagation is a major economic and public health concern due to the growing inefficacy of available antimicrobial agents to treat infectious disease. Wastewater treatment plants (WWTPs) play a vital role in minimizing the discharge of many water pollutants to the environment, including antibiotics and pathogenic microorganisms. However, WWTPs may also serve as breeding grounds and point sources for environmental dissemination of antibiotic resistance genes (ARGs) and ARB. In this talk, we will examine the behavior, replication, and fate of ARGs throughout the various processes, and discuss mechanisms that enhance their removal. For example, increasing the metabolic burden of resistance plasmid reproduction (e.g., higher growth rates, and low substrate and O2 availability) promotes ARG removal. Accordingly, manipulating WWTP operation variables (e.g., food-to-microorganism ratio or contact time in anaerobic digesters) may facilitate ARB control. Because some "superbugs" are relatively resistant to disinfection by chlorination, we will focus on the merits and limitations of biocontrol approaches using polyvalent (broad-host-range) bacteriophages, including proof-of-concept of indirect targeting of problematic bacteria, by knocking off its symbiotic and syntrophic species

Author: Pedro Alvarez, Rice University, United States

Biography:

Pedro J.J. Alvarez is the George R. Brown Professor of Civil and Environmental Engineering at Rice University, where he also serves as Director of the NSF ERC on Nanotechnology-Enabled Water Treatment (NEWT) and Director of the Rice Water Institute. His research interests include environmental implications and applications of nanotechnology, bioremediation, fate and transport of toxic chemicals, water footprint of biofuels, water treatment and reuse, and antibiotic resistance control. Pedro received the B. Eng. Degree in Civil Engineering from McGill University and MS and Ph.D. degrees in Environmental Engineering from the University of Michigan. He is the 2012 Clarke Prize laureate and also won the 2014 AAEES Grand Prize for Excellence in Environmental Engineering and Science, and the 2023 Outstanding Achievement Award by the China Chemical Society. Past honors include President of AEESP, the AEESP Frontiers in Research Award, the WEF McKee Medal for Groundwater Protection, the SERDP cleanup project of the year award, the Brown and Caldwell lifetime achievement award for site remediation, and various best paper awards with his students. Pedro currently serves as Executive Editor of Environmental Science and Technology. He was elected to the National Academy of Engineering for practical and pedagogical contributions to bioremediation and environmental nanotechnology.



Session 6

Abstract 235

Room 3

Phage Science, Therapeutics, and Research: The Public Health Agency of Canada's New Phage Therapy Program

Background and Aim: In response to the increased prevalence of antimicrobial resistance seen throughout the country, the Public Health Agency Canada (PHAC) has funded a 5-year pilot project to support phage therapy within Canadian clinics. The new Phage Science, Therapeutics, and Research (PhageSTAR) unit within the One Health Division of the National Microbiology Laboratory Branch aims to establish a phage library and screening processes to facilitate personalized phage mixtures tailored to isolates from individual patients' infections.

Procedure/Method: The PhageSTAR program will be implemented over a multi-year timeline, beginning with a comprehensive effort to create a phage library and to validate high-throughput screening methods with a goal of leveraging in-silico prediction. Environmental samples from ongoing national programs, notably wastewater, will be used to isolate additional phages targeting priority pathogens such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumonia. Once established, clinicians will then be able to submit clinical isolates for screening against the phage library to identify a combination of phages which can be formulated for patient administration.

Findings/Results: The PhageStar program has just launched and we are beginning engagements with stakeholders.

Implications/Applications: The program seeks to lessen the burden of antimicrobial resistance on the healthcare system and prolong the lifespan of antibacterials currently in circulation. The target of this program is to have successfully facilitated the treatment of 4 Canadians through phage therapy by the end of 2025 while establishing strong collaborations with both domestic and international partners. Furthermore, by engaging in both the regulatory and biopharmaceutical processes required for the creation of these therapeutics, the PhageSTAR pilot aims to significantly advance phage therapy and phage science for clinicians, researchers, and the biopharmaceutical industry alike.

Author: Michael Parcey, Public Health Agency of Canada, Canada

Co-Authors: Bharat, Amrita; Knox, Natalie; Mulvey, Michael

Back to the Program

Session 6

Abstract 134

Room 3

Phagotrophic protists preserve antibiotic-resistant opportunistic human pathogens in vegetable phyllosphere

Background and Aim: The phyllosphere of leafy greens is a reservoir of microbes, including antibiotic-resistant pathogens (ARPs), and a carrier for transmitting these microbes to humans. ARPs are also subjected to predation pressure by protists. However, our understanding of protist-ARP associations in the vegetable phyllosphere and their contribution to public health remains limited.

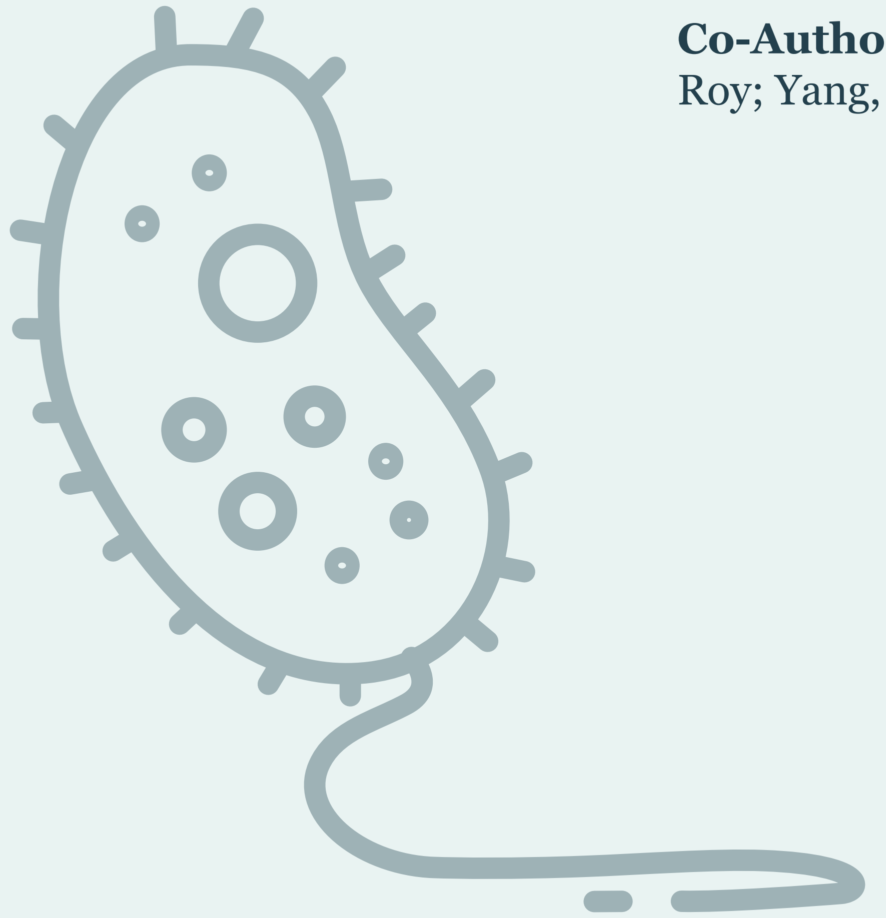
Method: Here, we characterized the protistan community and quantified human pathogen marker genes (HPMGs) in four vegetable species produced conventionally and organically from supermarkets in Xiamen, China, by High-throughput sequencing and High-throughput qPCR, respectively. Then we isolated endosymbionts of phyllosphere protists and identified their virulence, and resistance traits using whole genome sequencing.

Results: We report that potential human pathogens were detected in 90% of leafy greens, with Staphylococcus aureus and Klebsiella pneumoniae dominating. HPMGs abundance positively correlated with the diversity and relative abundance of phagotrophic protists. Colpoda were identified as key phagotrophic protists that positively linked to HPMG abundance and carried diverse multiple potential ARPs confirmed by isolated culture experiments. Whole genome sequencing further uncovered the coexistence and cotransfer potential of antibiotic resistance genes, virulence factors, and metal resistance genes in the genome of most isolated endosymbiont ARPs, which may increase their capacity to survive grazing. The isolated endosymbiont, e.g., Achromobacter xylosoxidans and Stenotrophomonas maltophilia had even higher virulence potential and lower antibiotic resistance potential compared to clinical strains.

Implications: These results suggest that phagotrophic protists are indicators and important niches for potential ARPs through internalization in the vegetable phyllosphere, thus posing a threat to the safety of leafy greens and human health in light of the “One Health” concept. Our study provides insights into the emergence and evolution of microbial pathogenicity and antibiotic resistance in natural environments.

Author: Chenshuo Lin, Institute of Urban Environment, Chinese Academy of Sciences, China

Co-Authors: Li, Li-Juan; Ren, Kexin; Zhou, Shu-Yi-Dan; Isabwe, Alain; Yang, Le-Yang; Neilson, Roy; Yang, Xiao-Ru; Cytryn, Eddie; Zhu, Yong-Guan



Session 6

Abstract 124

Room 3

Protozoa predation: a major pathway of E.coli removal in aerobic granular sludge

Background and Aim: During wastewater treatment, E.coli are removed effectively, inter alia through predation and attachment. However, little is known about the main removal pathway of E.coli in AGS systems, and how far the different sludge fractions of granulated sludge contribute to E.coli removal. In full-scale aerobic granular sludge (AGS) plants, larger granules (>2mm) dominate, while small granules and flocs typically coexist.

Procedure/Method: Predation and attachment batch experiments were conducted using 6 size classes of sludge under oxic and anoxic conditions. The concentration of E.coli in the liquid phase was measured during 24h to determine E.coli removal through predation or attachment. The counts and species composition of protozoa in different sludge fractions were observed microscopically after feeding fluorescently labeled E.coli.

Findings/Results: Compared with activated sludge systems, AGS showed a higher capacity for E.coli removal. Within the different sized granules, granules >1mm are major contributors to E. coli removal in AGS system, removing more than twice as much E.coli as small sludge fractions (<0.6mm). Predation is the main removal pathway of E.coli removal in granules, whereas attachment contributes more to E.coli removal in small sludge fractions and activated sludge. In granules, a large number of protozoa with strong E.coli predation capacity were observed. In small sludge fractions, in contrast, testate amoeba not feeding on E.coli dominate, and the counts of protozoa were lower. Both species composition and protozoan counts determine predation capacity across different-sized sludge, while there was only a minor effect of sludge size on attachment.

Implications/Applications: The results of this study provide information on the fate of E.coli within AGS systems and its relation with sludge size, and point to a significant role of protozoa in removal of E.coli.

Author: Zhaolu Feng, Wageningen University & Research, Netherlands

Co-Authors: Sutton, Nora; Schmitt, Heike; van Loosdrecht, Mark

Back to the Program

Session 6

Abstract 47

Room 3

Paper-based microfluidic device for the detection of antimicrobial resistance genes in drinking water

Background and Objective: The proliferation of antimicrobial resistance has been accelerated by the excessive use of antimicrobials. However, traditional detection methods are often time-consuming, costly, and require specialized technicians and advanced equipment. The purpose of this study is to develop an affordable, rapid, and convenient method for the detection of antibiotic resistance genes (ARGs) in drinking water by combining a paper-based microfluidic device and loop mediated isothermal amplification (LAMP) assay.

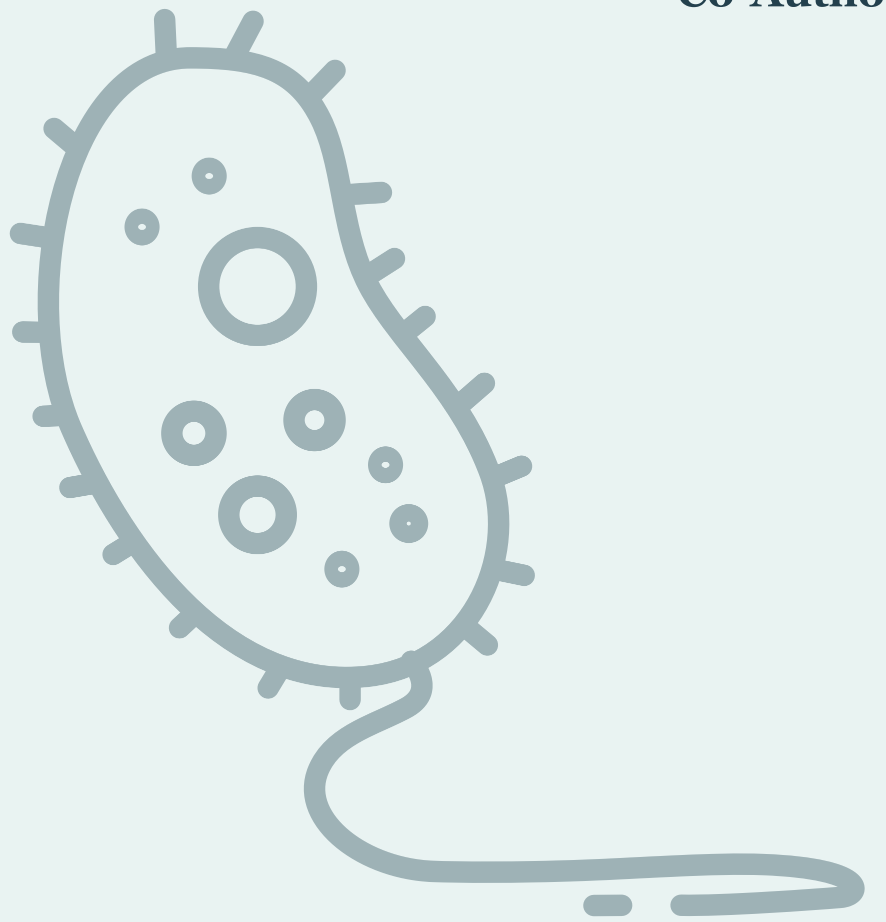
Procedure/Method: The design of the device was accomplished using CorelDRAW software and fabricated utilizing a wax printer. The LAMP assay was integrated into the device for the specific detection of the target ARGs. Additionally, a syringe-based system was developed for the enrichment of bacteria from water samples. The results were visualized using a portable UV torch and observed by the naked eye. Furthermore, the findings were validated through polymerase chain reaction (PCR) assay.

Findings/Results: The paper-based platform demonstrated the ability to concentrate, extract, and detect analytes in drinking water within a time frame of 2 hours. The optimal dimensions of the device, including pore size, channel width, and channel length, were determined to be 4.0 mm, 1.5 mm, and 2.5 mm, respectively. The LAMP assay successfully detected MCR-1, CTX-M-2, and NDM-1 at concentrations as low as 10 copies µL-1. In comparison to most existing methods, our assay exhibited favorable detection limits, comparable analysis time, and reduced cost.

Implications/Applications: The paper-based platform not only facilitates the prevention of ARG contamination, but also enables the prediction of disease outbreaks and reduces the risk of transmission. Moreover, the integration of the paper-based microfluidic device into field application modules opens new opportunities for on-site sample preparation and rapid detection of ARGs. This platform holds great promise for the routine monitoring of antimicrobial resistance in drinking water, particularly in resource-limited regions.

Author: Zhugen Yang, Cranfield University, United Kingdom

Co-Author: Pan, Yuwei



Tuesday, May 28 - 8:30-10:21

Back to the
Program

Keynote Speaker: Sabiha Essak

Session 7
Abstract 421
Room 3-4

The Polycrisis and AMR: Global Policy Fails the Vulnerable

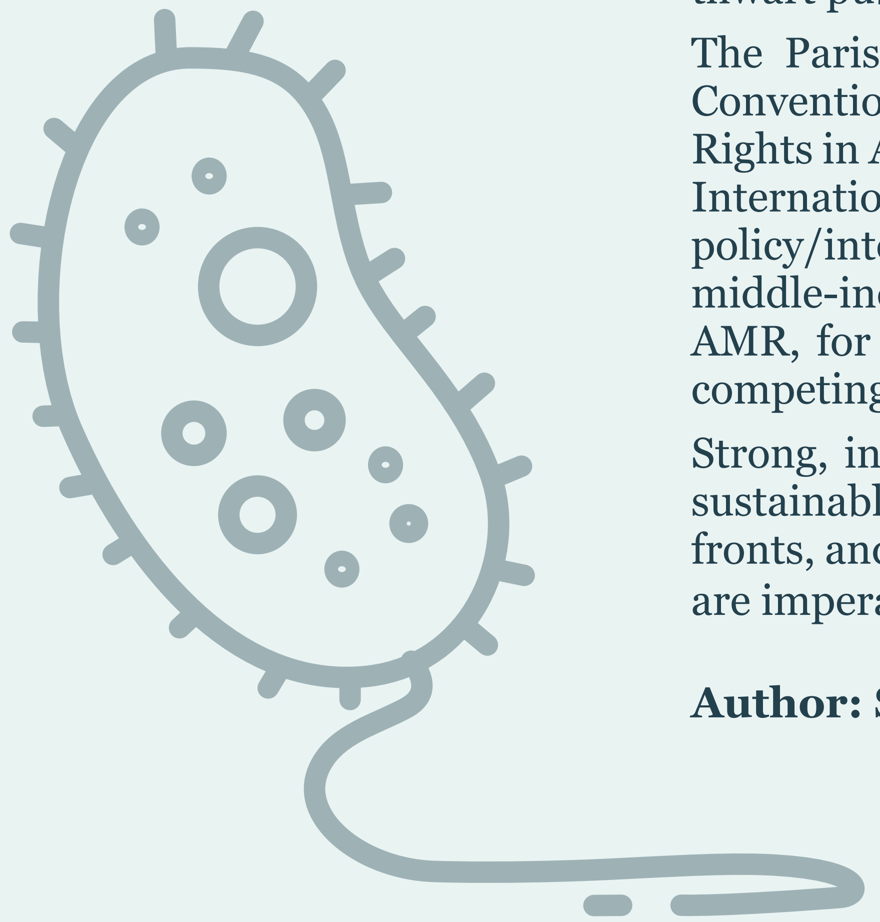
The polycrisis may be defined as “the sum total of all stressors affecting planetary health,” encompassing “biosphere stressors” such as climate change, biodiversity loss and ocean acidification, “societal stressors” such as poverty, malnutrition, supply chain vulnerabilities, war, conflict and displacement, “health stressors” such as endemic, epidemic and pandemic infectious diseases and chronic health diseases affecting humans, animals and plants within failing One Health systems, and “technological stressors” such as automation, artificial intelligence, and cyber threats , . The interaction of these disparate stressors create the perfect storm for antimicrobial resistance (AMR).

Climate change is a threat multiplier of AMR. Increasing temperatures have been associated with increasing antibiotic resistance in common pathogens, horizontal gene transfer is facilitated at higher temperatures amongst soil bacteria and fungi, and, high temperatures have resulted in the release of ancient AMR genes from thawing permafrost. Simultaneously, the global demand for cheaper protein has resulted in a proliferation of intensive food animal production where antibiotics are used for growth promotion, prophylaxis and metaphylaxis in lieu of biosecurity. Livestock farming also generates substantial greenhouse gases exacerbating both climate change and AMR. War, conflict and displacement result in high population densities with inadequate access to water, sanitation and hygiene infrastructure and sub-optimal vaccination coverage, facilitating the transmission of water- and air-borne infections that are worsened by lack of access to appropriate diagnostics, therapeutics and healthcare in general. Infections with drug-resistant pathogens have been extensively reported in the survivors and perpetrators of armed conflict. Poverty and consequential malnutrition increase the risk of infectious diseases. Prophylactic antibiotic treatment of severe acute malnutrition and mass administration of antibiotics carry the risk of selection pressure for the emergence/escalation of AMR. Access and availability to therapeutics, diagnostics and vaccines are vulnerable to fragile supply chains especially in pandemic or global drug shortage situations and oftentimes unaffordable because of intellectual property rights. Misinformation and disinformation on social media and increasingly on mainstream media thwart public health efforts to address the polycrisis and AMR.

The Paris Agreement, a legally binding international treaty on climate change, the Geneva Convention on International Humanitarian Law, the International Legal Protection of Human Rights in Armed Conflict, the Food Aid Convention, the Convention on the Rights of the Child, the International Health Regulations and the Global Action Plan on AMR are all examples of global policy/international law that have, to a large extent, failed the vulnerable, particularly in low and middle-income countries (LMICs) that bear the disproportionate burden of the polycrisis and AMR, for lack of enforcement and political will, resource constraints, and, equally urgent and competing priorities.

Strong, innovative political leadership to resolve, not just manage these crises, innovative and sustainable funding models to facilitate the implementation of evidence-based solutions on multiple fronts, and an enforcement apparatus to ensure compliance to global policy and international law are imperative!

Author: Sabiha Essack, University of KwaZulu-Natal, South Africa



Biography:

Sabiha Essack is the South African Research Chair in Antibiotic Resistance and One Health and Professor in Pharmaceutical Sciences at the University of KwaZulu-Natal. She is the Chair of the WHO Strategic and Technical Advisory Group for Antimicrobial Resistance (STAG-AMR) and co-chair of the Quadripartite Technical Group on Integrated Surveillance. Professor Essack serves as the Senior Implementation Research Advisor to the International Centre for Antimicrobial Resistance Solutions (ICARS) in Denmark and a member of the Scientific Advisory Board of the Joint Programming Initiative on AMR (JPIAMR). She is chairperson of the Global Respiratory Infection Partnership (GRIP), she serves on the Advisory Board of the Combating Antibiotic Resistant Bacteria Biopharmaceutical Accelerator (CARB-X) and the Fleming Fund Expert Advisory Group. Her research focuses on the molecular epidemiology of AMR using next generation sequencing and bioinformatics as well as One Health systems strengthening in the context of AMR.

ORCID: https://orcid.org/0000-0003-3357-2761
Website: http://sabihaessack.ukzn.ac.za

Tuesday, May 28 - 8:30-10:21

Back to the Program

Session 7
Abstract 362
Room 3-4

Estimates of Human Exposure to Antimicrobial Resistance Foodborne Pathogens and the Resulting Burden of Illness

Background and Aim: Antimicrobial resistance (AMR) is a threat to global population health. The agriculture sector is a major user of antimicrobials, accounting for approximately 82% of antimicrobial use (AMU) in Canada. To address the potential of AMR in the food supply chain, the poultry industry in Canada initiated an AMU Reduction Strategy, eliminating the preventive use of Category I antimicrobials in 2014 and eliminating the preventive use of Category II antimicrobials in 2018. This study investigates the economic consequences of AMR in foodborne pathogens, focusing on Salmonella spp. and Campylobacter spp. in Canadian poultry. The objectives were to assess the human burden of illness associated with antimicrobial-resistant foodborne bacteria and quantify the benefits of AMU reduction policy interventions in the poultry sector from 2013 to 2019.

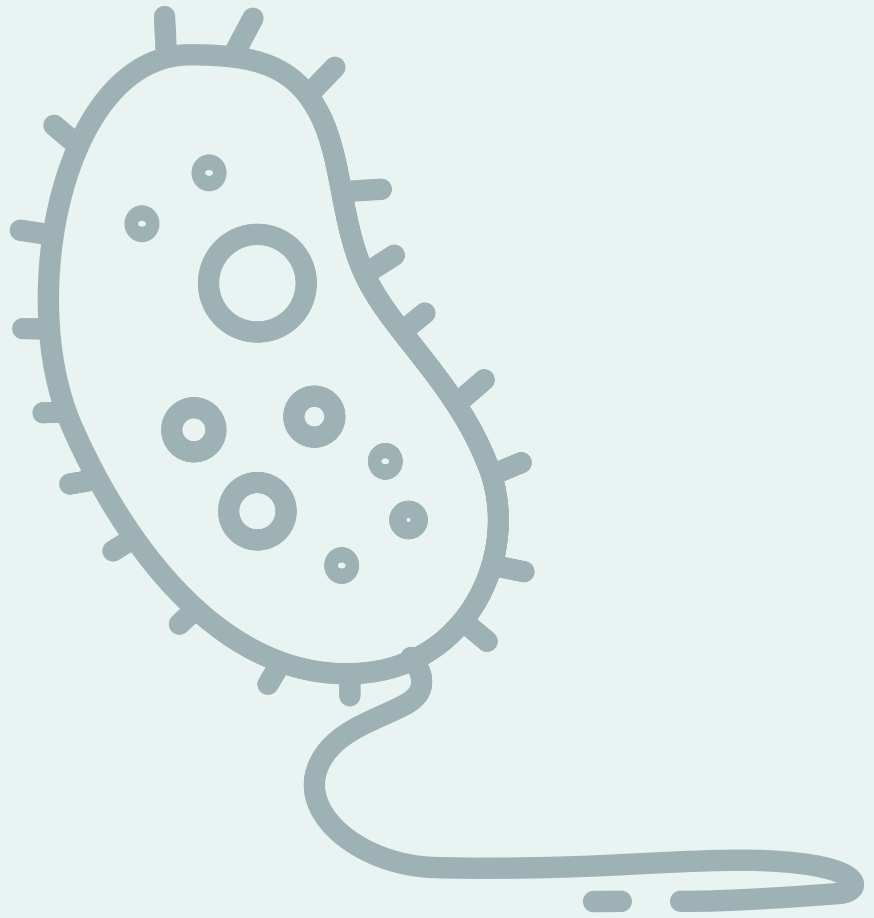
Method: This study used a Markov Chain Monte Carlo Simulation to model the progression of Salmonella spp. and Campylobacter spp. infections, distinguishing between AMR and susceptible strains across various health states. Transition probabilities were generated based on public health surveillance data and existing literature that mapped the sequential flow from one health state to another, with each state assigned specific health utility values and costs.

Findings: Preliminary results indicate that resistant infections with foodborne pathogens impose a larger economic burden, with higher medical costs and lower Quality-Adjusted Life-Years (QALYs) compared with susceptible infections. The study highlights that reducing AMU in animal production, in this analysis in broiler chickens, reduces AMR, decreases health care costs, and can potentially yield macroeconomic benefits, including increased industrial output and GDP.

Implications: These findings underscore the importance of integrated One Health approaches in addressing AMR. They provide additional information for policy decision-making and assessment of alternative policy choices.

Author: Yarina You, McGill University, Canada

Co-Authors: Reid-Smith, Richard; Parmley, Jane; Mukhopadhyay, Kakali; Thomassin, Paul



Session 7
Abstract 400
Room 3-4

Antimicrobial resistance National Action Plan goals translating into federal policies: A comparative analysis between the United States and Canada

Background and Aim: The dimensionality and scale of antimicrobial resistance (AMR) demands comprehensive policy solutions and global coordination to avoid a public health crisis in the coming decades. Despite over 130 countries developing AMR National Action Plans (NAPs), the translation of NAP goals into national policies can be lacking as it requires strong implementation efforts. Therefore, our study investigates NAP implementation efforts and international coordination by focusing on the United States and Canada. Our objective is to understand how effectively each country implements its NAP goals through federal legislative and regulatory policies via a comparative analysis.

Procedure/Method: We developed an evaluative framework to assess NAP goals for each country based on specific criteria, including issue identification, policy recommendations, implementation strategies, monitoring, and evaluation. We then systematically identified federal AMR-related legislation and regulations across sectors (i.e., wastewater, healthcare, animal agriculture, pharmaceutical development), allowing us to assess the efficacy of AMR policy implementation in relation to NAP goals and draw comparisons between countries.

Findings/Results: Initial results comparing the first iteration of American and Canadian NAPs showed that the U.S. had more explicit goals and assigned tasks to specific federal agencies, with a primary focus on healthcare, data management, and its international role. Canada offered more definitive policy recommendations and expanded its scope to include education, agricultural industries, and pharmaceutical development.

Implications/Applications: This study addresses two critically important aspects necessary to combat AMR globally: NAP implementation and international alignment. Our results highlight national strategies for developing and executing strong implementation plans that embed NAP goals into national policy agendas. We also address shortcomings, such as the lack of policies around wastewater surveillance and monitoring, as well as opportunities for effective translation from NAP goals into federal policies.

Author: Lucas Goodman, Virginia Tech, United States

Co-Author: Schenk, Todd

Tuesday, May 28 - 8:30-10:21

Back to the Program

Session 7

Abstract 346

Room 3-4

Combating the Spread of Antibiotic Resistance Negotiation Simulation: Using Serious Games to Simulate Policy Deliberation

Background and Aim: Antimicrobial resistance represents a One-Health issue, indicating that the drivers and potential solutions are found in many sectors. Policy development in the field requires the engagement of stakeholders from government agencies and interest groups in order to create informed, broadly supported, and effective policies. This mixed-methods study examines how serious games can be used to teach both stakeholders and researchers about the importance of antimicrobial resistance, how stakeholders and their values influence the policy process, and how science is more or less effectively used in policy negotiations.

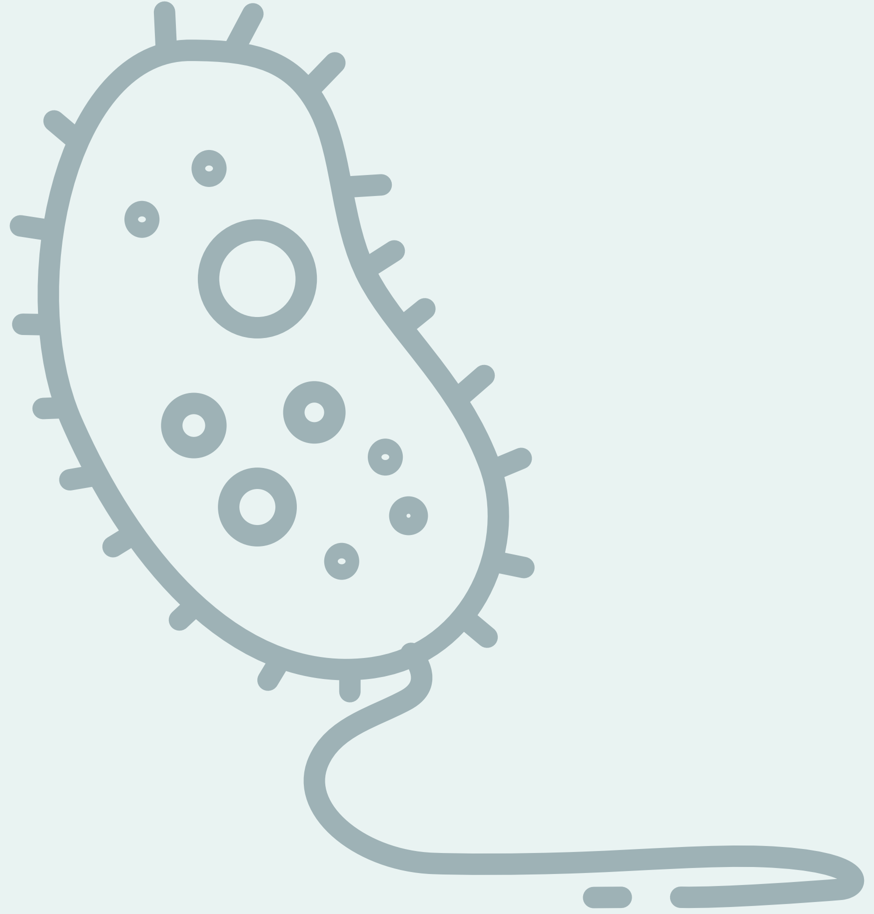
Procedure and Method: Using social learning theory, we developed a multi-role negotiation-style serious game focused on developing policy recommendations based on given information and assigned stakeholder interests and values. We surveyed the players of the game to understand the effectiveness of the exercise against six different learning objectives using a pre/post-survey design. The game was immediately followed by a semi-structured focus group in which the participants were asked to reflect on the experience. We generated descriptive statistics and a Wilcoxon matched pairs test comparing pre- and post-exercise surveys, and coding and analysis of the focus group.

Findings/Results: It was found that players of the game learned that the interests and values of stakeholders play a significant role in policy deliberation. Participants noted that they felt that the interests should play less of a role, and consequently using more scientific information when forming policy recommendations. The players felt that the game was effective in teaching how policy deliberations can be impacted through the use of scientific information and the influence that different stakeholders and their values can have.

Implications/Applications: The findings of this study demonstrate how serious games are effective in cross-disciplinary training, forming more effective collaborations to combat antimicrobial resistance and promote evidence-based decision-making.

Author: Rebekah Riddle, Virginia Tech, United States

Co-Author: Schenk, Todd



Tuesday, May 28 - 11:00-12:30

Back to the Program

Session 8

Abstract 434

Room 4

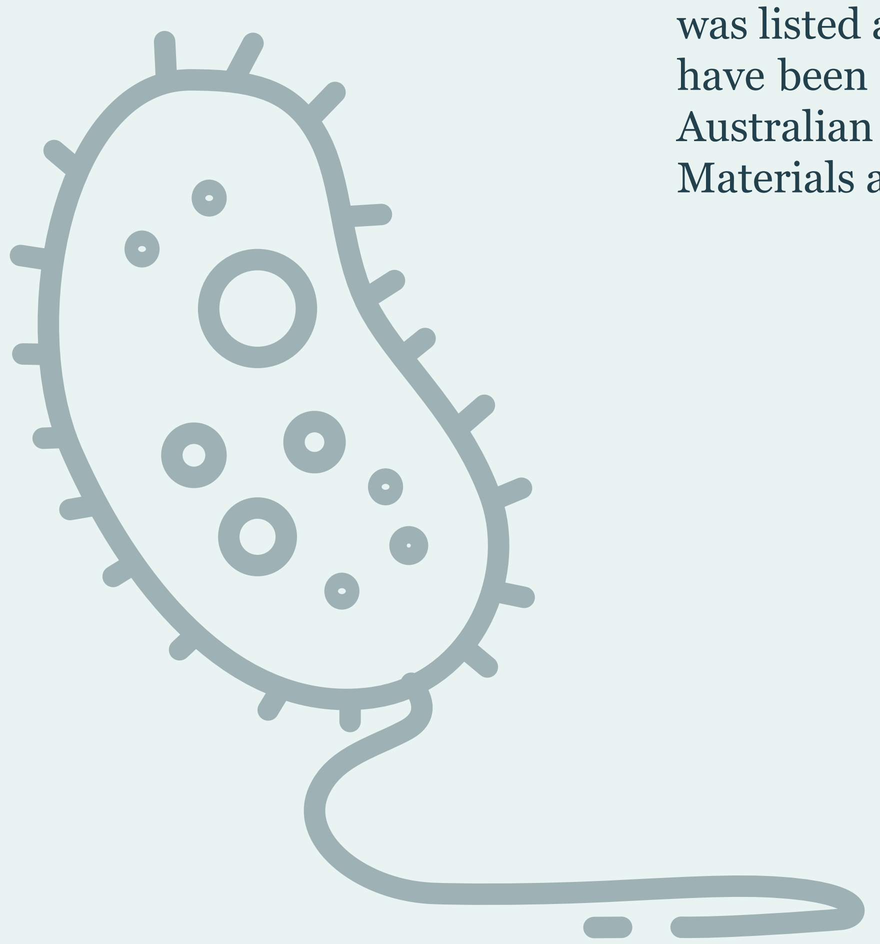
Emergence, evolution and spread of antibiotic resistance associated with non-antibiotic drugs

Although non-antibiotic pharmaceuticals are widely used by society with 95% of the drugs market share, little is known about whether non-antibiotic pharmaceuticals cause or accelerate the dissemination of antibiotic resistance. We have been focusing on exploring the interactions between non-antibiotic pharmaceuticals and the transmission of antibiotic resistance. Multiple bacterial models (including mutation, evolution, and horizontal gene transfer) in conjunction with in vivo animal gut microbiota assays were established to investigate antibiotic-like effects of commonly-used non-antibiotic drugs (e.g. antidepressants, anti-inflammatories and lipid-lowering drugs) on the emergence and spread of antibiotic resistance. We found that these non-antibiotic pharmaceuticals not only induce the emergence of antibiotic resistance through mutation, but also promote the dissemination of antibiotic resistance via horizontal gene transfer. This was demonstrated in both pure-culture bacterial models and mixed-culture environmental samples, as well as animal gut microbiota and human gut simulators. Several common mechanisms, including over-generation of reactive oxygen species, cell membrane variation, and stress level elevation, are playing key roles. Considering the high-consumption and wide-detection of non-antibiotic pharmaceuticals, these findings emphasize concerns of non-antibiotic pharmaceuticals for the emergence and spread of antibiotic resistance in human/ animal gut and the environment, and advance our current understandings of the dissemination of antibiotic resistance.

Author: Jianhua Guo, The University of Queensland, Australia

Biography:

Professor Jianhua Guo is the Deputy Director- Research of the Australian Centre for Water and Environmental Biotechnology at The University of Queensland, Australia. Dr Guo and his team found that non-antibiotic pharmaceuticals not only induce the evolution of multi-drug resistant bacteria via genetic mutation, but also promote the spread of antibiotic resistance through horizontal gene transfer. He has to date published over 250 peer-reviewed papers since 2007. He was listed as Clarivate Highly Cited Researcher for 2023. His research output and achievements have been recognized through national and international awards or fellowship, including 2017 Australian Research Council Future Fellowship. He is an Editor of the Journal of Hazardous Materials and an Associate Editor of Water Research.



Session 8

Abstract 184

Room 4

Dynamics of class 1 integrons in wastewater treatment plants and hospital wastewater

Background and Aim: Class 1 integron-integrase (intI1) gene is used as an indicator for ARB, as it can carry gene cassette encoding antibiotic resistance. However, intI1 monitoring could not distinguish human-infected and not human-infected intI1 genes. This can lead over estimation of human health risk. In this study, we measured intI1 gene concentrations in intracellular DNA (iDNA) and extracellular DNA (eDNA) in wastewater treatment plants (WWTPs) and hospital wastewater (HW), by further classifying human-infected intI1 into those with/without gene cassette using aintI1 and eintI1 assays, to estimate the fate of ARB and ARGs in detail.

Methods : Influent, secondary effluent, and final effluent samples of two WWTPs were collected in June 2022 and May 2023, and HW samples were collected once a month from April 2021 to June 2022. Fifty-milliliters of the samples were filtered using a 0.22μm membrane and iDNA was extracted from the filters while eDNA was recovered from the filtrate by ethanol precipitation. The gene concentrations were measured by qPCR or Droplet Digital PCR.

Results & Discussion: In HW, concentrations of 16S rRNA, intI1, and aintI1 in iDNA were stable around 10 log10, 9 log10, and 9 log10 copies/L, respectively, while eintI1 fluctuated from about 6 log10 to 9 log10 copies/L. The aintI1 concentration was always higher than eintI1, probably due to the selective pressure by high concentrations of antibiotics and other chemicals in HW. For 2 WWTPs, similar trend was observed for iDNA, i.e., a reduction of about 2 - 3 log10 for each target during the treatment process. However, the gene concentrations in the effluent increased probably due to cell damage during chlorine disinfection, and more than 4.5 log10 copies/L intI1 genes were released into the environment as eDNA. Therefore, occurrence and proliferation of ARB by natural transformation of ARGs to non-ARB should be further evaluated.

Author: Kaho Misawa, Kitasato University, Japan

Co-Authors: Yamamoto, Tomoya; Amarasiri, Mohan; Maehana, Shotaro; Furukawa, Takashi; Sano, Daisuke; Sei, Kazunari

Back to the Program

Session 8

Abstract 9

Room 4

Evolution dynamics of antimicrobial resistance under chlorination disinfection

Background: Chlorination is a widely used disinfection technology to prevent and control outbreaks of waterborne diseases in drinking water. Previous studies have reported that chlorination can enrich antibiotic resistance genes (ARGs) and promote ARG transfer between bacterial species. However, it is unknown whether chlorination could drive the long-term evolution of resistance to chlorine disinfectant and different antibiotics across bacterial species such as waterborne pathogens.

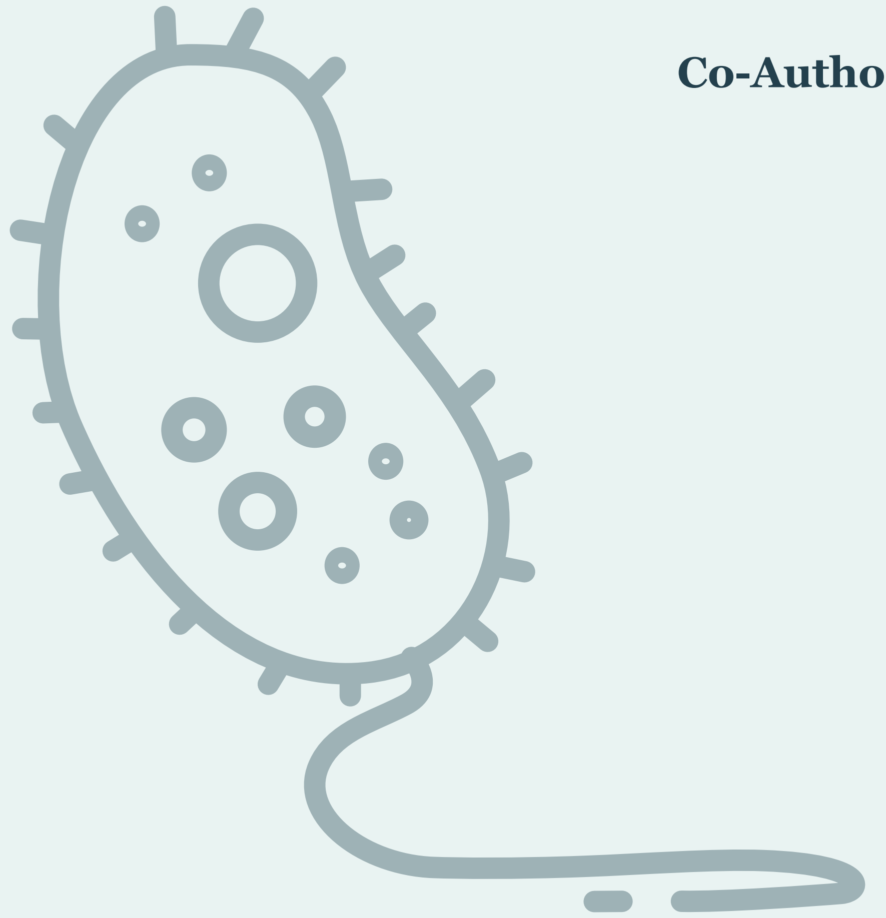
Method: We conducted stepwise chlorine exposure experiments with a model strain Escherichia coli and two opportunistic pathogens Pseudomonas aeruginosa and Legionella pneumophila. Minimum inhibitory concentrations (MICs), oxidative stress, and whole genome DNA and mRNA sequencing were analysed.

Results: Over a period of ~30 days, resistance levels increased dramatically (e.g. MIC values of E. coli >1,000-fold against colistin and trimethoprim), with parallel populations of three strains showing similar phenotypic trajectories. The mutation ratios significantly increased: >106 folds in E. coli (colistin), >100 folds in both P. aeruginosa (tetracycline) and L. pneumophila (tetracycline and ciprofloxacin). Resistance patterns were delayed by exposure to low chlorine dosages (0.5 and 1 mg/L), but they eventually manifested at the same levels when exposed to high chlorine dosages (2.0 and 4.0 mg/L). The isolated resistant colonies of three strains well maintained their acquired multi-drug resistance >200 generations and showed survival advantages against their parents in the presence of chlorine. Such resistance development could be due to chlorine-induced oxidative stress. Whole genome sequencing of both the populations and the evolved isolates identified mutations shared in resistance (e.g. enhanced stress response and efflux pump) to multiple antibiotics.

Implications: Our findings provide solid evidence of chlorine-associated antimicrobial resistance. Given that chlorination disinfection is widely applied in water and drinking water treatment plants and that the prevalence of water-borne pathogens, our findings also highlight the need to re-evaluate the potential health risks linked to this disinfection technology.

Author: Zhigang Yu, The University of Queensland, Australia

Co-Author: Guo, jianhua



Session 8

Abstract 322

Room 4

The Role of Bacteriophages as Vectors of Antimicrobial Resistance in Wastewater Treatment Plants is Very Limited: A qPCR and Metagenomics-Based Study

Background and aim: The literature on the role of bacteriophages (transduction) in the dissemination of antibiotic resistance is very limited and controversial. This study aims to provide insights into the role of mobile genetic elements (MGEs), with a special emphasis on bacteriophages as potential carriers of ARGs, in the dissemination of antibiotic resistance and investigate changes in resistome, bacterial and bacteriophage profiles within WWTPs using metagenomics and qPCR assays.

Methods: Wastewater and sludge samples were collected from different processes in two WWTPs (Winnipeg, Canada) and processed to separate and concentrate bacterial and bacteriophage fractions using physical and chemical techniques. A total of 44 extracted DNA samples were sequenced, which produced 35 million reads per sample on average. Up-to-date microbial bioinformatics tools and databases were employed to process reads, and identify bacterial and bacteriophage communities, prophages, ARGs, hosts of ARGs, MGEs and hosts of bacteriophages using command-line on HPC servers. qPCR assays were conducted for bacterial and bacteriophage fractions by targeting several common ARGs and 16S rRNA.

Results: Only one ARG, lnuC, was detected in over 500,000 phage contigs. Although some ARGs were detected with qPCR in bacteriophage fractions, these ARGs were closely associated with the bacterial DNA contamination in bacteriophage fractions and other MGEs through bioinformatics, qPCR assays and statistical analyses. Few peptide and beta-lactam resistance genes were found to be prophage-related. Diversity analyses showed that the profiles of bacteria, bacteriophages, hosts of bacteriophages and ARGs in WWTPs were significantly different. Most of the ARGs (>60%) in terms of abundance were associated with mobile genetic elements (except bacteriophages).

Implications: The results indicated that bacteriophages rarely carry ARGs, which negates some other studies emphasizing the role of bacteriophages as vectors of ARGs. The abundance and diversity of ARGs were lower in effluent samples, indicating the efficiency of WWTPs in removing ARGs.

Author: Kadir Yanac, University of Manitoba, Canada

Co-Authors: Yuan, Qiuyan; Uyaguari, Miguel

Tuesday, May 28 - 11:00-12:30

Back to the Program

Session 9

Abstract

Room 3

TBC

Session 9

Abstract 398

Room 3

Assessment of risks associated with antibiotic-resistant E. coli through consumption of produce irrigated with reclaimed wastewater

Background and Aim: Antimicrobial resistance (AMR) is one of the greatest public health threats facing humanity. Within the approach of "One Health," there is growing focus on the environmental dimensions, though this extent is challenging to quantify. Agricultural irrigation using reclaimed wastewater (RWW) represents a sustainable practice to meet the ever-increasing water stress around the world. However, the occurrence of antibiotics and antibiotic-resistant bacteria (ARB) in RWW and their risks are under-studied. Detectability of fecal bacteria and AMR genes on irrigated produce and their proliferation under gut-like conditions suggests that there are associated risks. This study applies a novel approach within the quantitative microbial risk assessment (QMRA) framework accounting for: 1) the presence of ARB in irrigation water and on produce, 2) conditions which may contribute to horizontal gene transfer, and 3) risk outcomes based on subinhibitory antibiotics and mixed doses of susceptible and resistant bacteria.

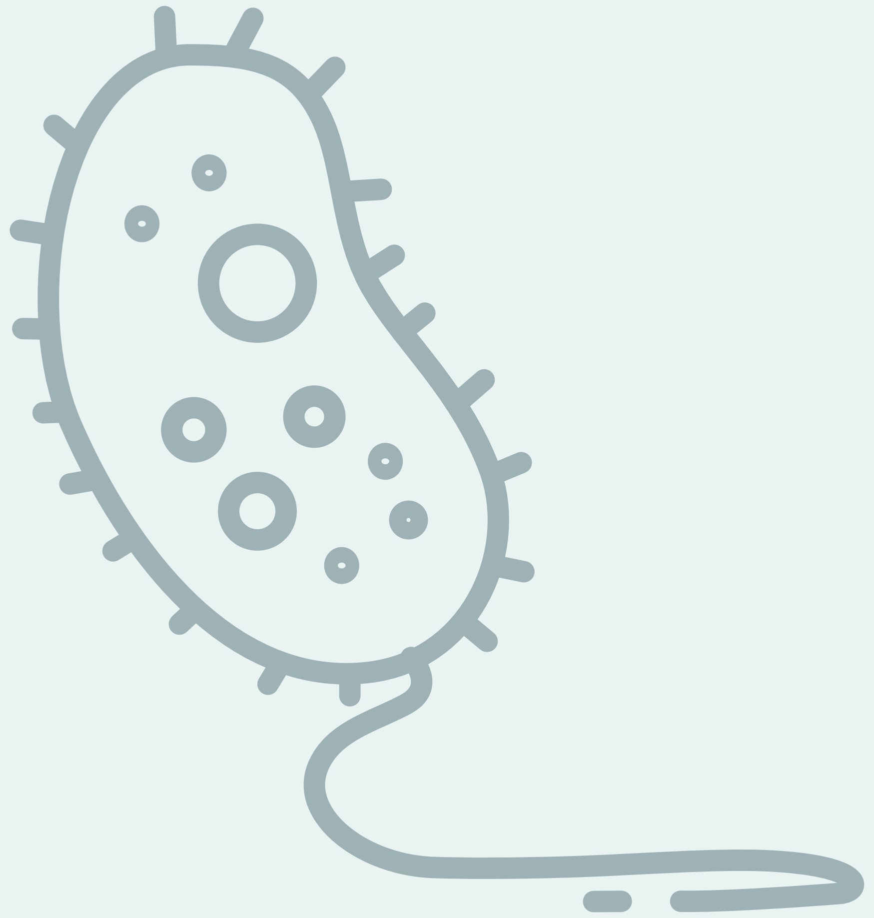
Procedure/Method: A risk model based on the QMRA framework and the exposure through consumption of RWW irrigated produce is quantified. Based on the concept of simple death modeling, the dose-response is predicted, with variability regarding the presence of residual/subinhibitory antibiotics and impacts of horizontal gene transfer on a population dynamics sub-model. Health data are reviewed, and gaps identified for reality-checking risk models.

Findings/Results: Risk results show that the presence of residual antibiotic in human body reduced the overall risk probabilities of infections but selectively enhanced the survival of ARB in comparison to their susceptible counterparts, which resulted in antibiotic untreatable infection.

Implications/Applications: This new approach using the traditional QMRA framework can be utilized by regulators and stakeholders to reduce the risks of antibiotic resistant fecal bacteria from and establish proper guidelines for safe reuse of treated wastewater. In addition, the workflow is readily adaptable to other exposure scenarios or pathogens where data is available.

Author: Hunter Quon, Arizona State University, United States

Co-Author: Hamilton, Kerry



Back to the Program

Session 9

Abstract 372

Room 3

Combining field studies, data and modelling to inform a Bayesian Network of risk to human health from environmental AMR

Background and Aim: Antimicrobial resistant bacteria (ARBs) are transported from different sources along various pathways through the environment. Antibiotics are also transported. During transport, ARBs may grow, die or become diluted along the way. Antimicrobial resistance genes (ARGs) can be transferred horizontally, depending on local environmental conditions. Selection for resistance depends on the concentrations of (mixtures of) selective agents. Our aim is to understand this complex combination of processes and conditions that ultimately affect the exposure of humans to ARBs and the associated risks and to identify potential mitigations.

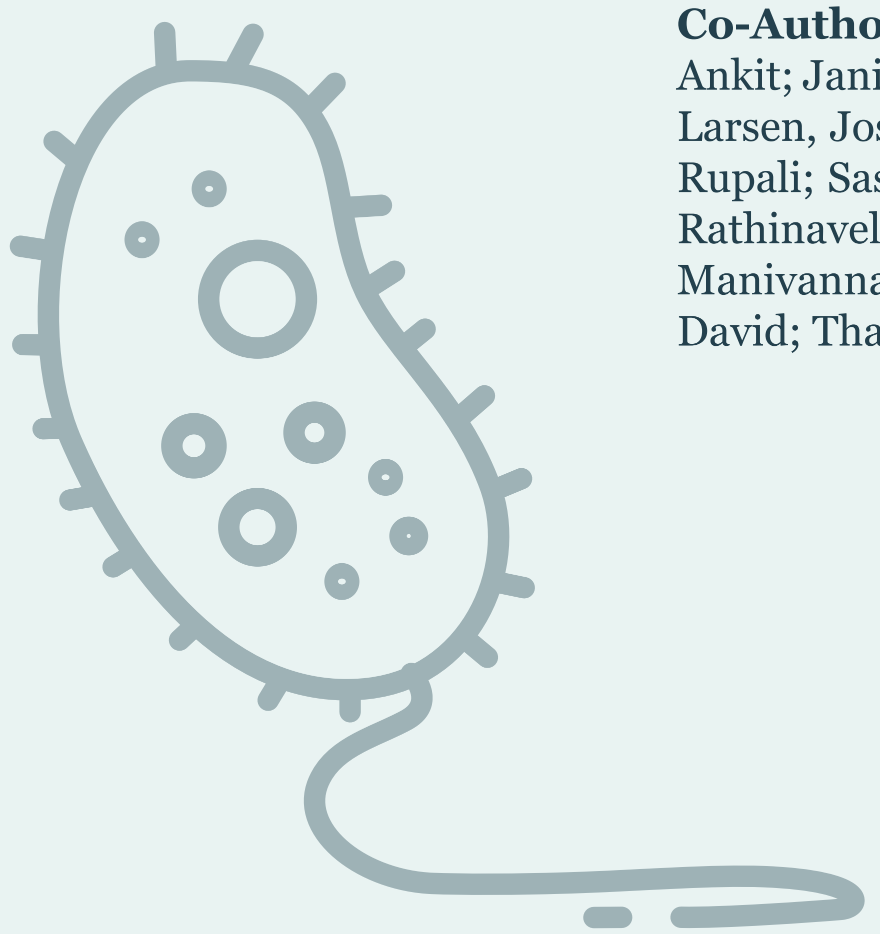
Procedure: Our Indo-UK project AMRflows seeks to combine observations, experiments, data and mathematical modelling to inform a Bayesian Network (BN) that captures the transport, dynamics and dilution of ARBs along the exposure pathways to assess risk to human health.

Findings: Sewage pollution from the large city Hyderabad was the main driver of AMR in the Musi river, masking any input from antibiotic production facilities. The Adyar river flowing through another large city, Chennai, is already too polluted when it enters the city to discern an effect of sewage pollution from the city. Another difference to the Musi is that the wet season increases ARG abundance. The IncP plasmid pKJK5 can transfer in nutrient poor media mimicking river conditions, but only at low rates. Zebrafish larvae develop deformations when incubated in Musi river water but the presence of E. coli (sensitive or resistant) has no effect. Predicting concentrations of antibiotics in the rivers from sales data and hydrological/hydraulic modelling of dilution is possible but validation proves challenging.

Implications/Applications: Using our data, the literature and predictions from our modelling, we constructed a Bayesian Network assessing the risk to human health from infection by ARBs. Importantly, any uncertainty in inputs percolates through the network to generate uncertainty in the output – the risk.

Author: Jan-Ulrich Kreft, University of Birmingham, United Kingdom

Co-Authors: Troldborg, Mads; Hough, Rupert; Uluseker, Cansu; Safi, Wahidullah Hakim; Modi, Ankit; Janivara Chandregowda, Chaitanya; Mohapatra, Pranab Kumar; Kuiry, Soumendra Nath; Larsen, Joshua; Sonkar, Vikas; Kashyap, Arun; Chandrakalabai Jambu, Sangeetha; Srivastava, Rupali; Sasidharan, Sai Sugitha; Rengan, Aravind Kumar; Methale Velakkath Paramba, Thara; Rathinavelu, Sasikaladevi; Viswanathan, Arathy; Vaidyanathan, Rama; Nambi, Indumathi Manivannan; Pallares Vega, Rebeca; Ovis Sanchez, Julian Osvaldo; Harwood, Colin; Graham, David; Thatikonda, Shashidhar



Session 9

Abstract 287

Room 3

MetaCompare 2.0: Differential ranking of ecological and human health resistome risks

Background and Aim: Metagenomic sequencing holds promise as a means to achieve integrated surveillance of antimicrobial resistance. However, there is a need for relevant and consistent analytical approaches for comparison of metagenomes across humans, animals, and the environment. Recently, the concept of “resistome risk” has been advanced, which is a function of the proportion of contigs that contain antibiotic resistance genes (ARGs), mobile genetic elements (MGEs), and pathogen gene markers. MetaCompare 2.0 further refines this concept, developing distinct scores representing human health resistome risk (HHRR) and ecological resistome risk (ERR).

Procedure/Method: In the MetaCompare 2.0, HHRR scores are a function of the subset of ARGs that are of acute human health concern, MGEs, and ESKAPE pathogens that are co-occurring in contigs with those ARGs, while the ERR more broadly includes all ARGs, co-occurring MGEs, and human pathogens. We evaluated the robustness of the pipeline across different environments and sequencing characteristics through analysis of both in-house and publicly available metagenomes.

Findings/Results: Our evaluations demonstrate that MetaCompare 2.0 scores are consistently higher in environments affected by contamination or other stressors. The risk scores were robust and comparable across a range of sequencing depth, contig count, library coverage, and assembly algorithms. The pipeline is available at http://metacompare.cs.vt.edu/, providing an easy-to-use interface for visualizing results.

Implications/Applications: MetaCompare 2.0 can help identify and differentiate hot spots of concern for human exposure and for the evolution and spread of AMR.

Author: Monjura Afrin Rumi, Virginia Tech, United States

Co-Authors: Oh, Min; Davis, Benjamin C.; Juvekar, Adheesh; Brown, Connor L.; Vikesland, Peter J.; Pruden, Amy; Zhang, Liqing

Back to the Program

Session 10
Abstract 424
Room 4

Antimicrobial resistance (AMR) in soil-plant systems

Background and aim: Soil is a rich reservoir of antimicrobial resistance (AMR) genes, both intrinsic and extrinsic. The movement of AMR (both resistance genes and bacteria harboring these genes) within soil-plant systems is a common pathway of human exposure to environmental AMR.

Methods: In a series of experiments, we have been investigating three aspects of AMR movement: 1) the extent of microbial movement through the xylem of plant using tomato as a test plant; 2) how environmental factors, such as pollution and use of organic fertilizers, affect the movement of AMR from soil to plant tissues (including seeds); 3) through sampling market fresh produce, we used metagenomics and cultivation approaches to characterize AMR in the phyllosphere.

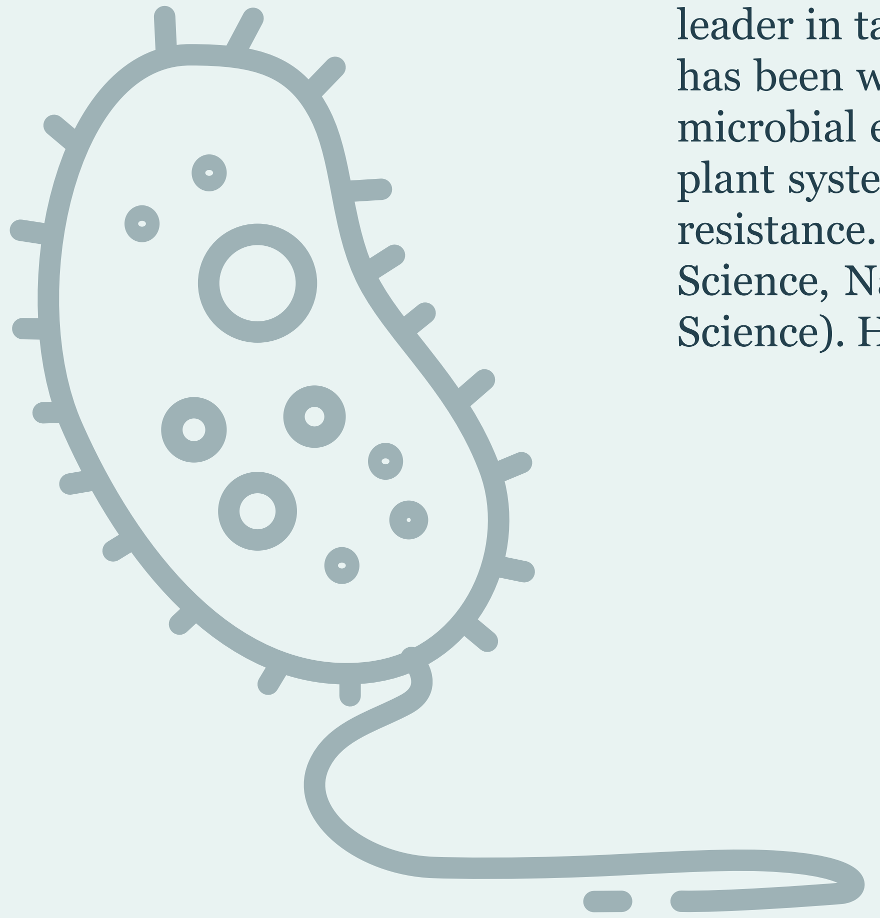
Results: We found that the xylem that transport water and solutes from the root to shoot harbors diverse bacteria and thus AMR. Manure application can have significant impacts on accumulation of AMR in plants (leaves and seeds). Combined exposure to chemical mixtures changed the profiles of AMR genes and vilurence factor genes (VFGs) by decreasing microbial diversity in phyllosphere. Market survey showed, that despite large geographic and climatic differences across sites, the specific recruitment of AMR in the vegetable phyllosphere is robust across host genotypes.

Implications: Understanding the dynamic of AMR in soil-plant systems is essential in quantitatively assessing the health risks of environmental (soil in particular) AMR to human health. Investigations on the driving factors that affect the movement of AMR in soil-plant systems will help develop solutions to mitigate the risks associated with soil contamination of AMR.

Author: Yong-Guan Zhu, Chinese Academy of Sciences, China

Biography:

Professor Yongguan (Yong-Guan) Zhu, Academician of the Chinese Academy of Sciences (CAS), Fellow of TWAS (The World Academy of Sciences), Fellow of International Science Council (ISC), professor of environmental science, is currently the Director General of the Research Center for Eco-environmental Sciences, Chinese Academy of Sciences, and formerly Director General of the Institute of Urban Environment, Chinese Academy of Sciences (2009-2018). Professor Zhu is a leader in taking multi-scale and multi-disciplinary approaches to environmental problems, and has been working on environmental health and wellbeing related to pollution, biodiversity and microbial ecology, particularly in biogeochemistry of arsenic, nitrogen and phosphorus in soil-plant systems, soil microbiome and soil health, and environmental dimension of antimicrobial resistance. Professor Zhu has published over 600 papers in international journals (such as Science, Nature, PNAS, Nature Microbiology, Nature Plants), with an H-index of 121 (Web of Science). He was selected as a Web of Science Highly Cited Researcher (2016-2023).



Session 10
Abstract 395
Room 4

Enhancing the Management of Antimicrobial Resistance (EMAR) in Low- and Middle-Income Countries: A Focus on Fiji

Background and Aim: Many low- and middle-income countries (LMICs), including the Fiji Islands, face high vulnerability to the antibiotic resistance crisis. This vulnerability stems from various factors, such as limited surveillance and diagnosis capabilities, as well as insufficient infrastructure for managing human and animal waste. This inadequacy contributes to increased environmental emissions of antibiotic-resistant bacteria and residual antibiotics. Within the EMAR project, we have designed a multifaceted 'One Health' approach to AMR surveillance, specifically tailored for Fiji.

Procedure/Method: The genomic surveillance program, utilizing whole-genome sequencing (WGS), metagenomics, and qPCR, aimed to understand the spread and evolution of AMR. This enabled the tracing of clonal expansion and transmission of existing and emerging pathogens, as well as mobile genetic elements (MGEs) that capture and spread AMR. To tackle this, we collected faeces, soils, waters, and food samples from urban and rural hospitals, wastewater treatment plants, abattoirs, farms, markets, recreational areas, open dumps, and rivers in Viti Levu, the largest and most populated island in Fiji.

Findings/Results: Metagenomics and qPCR analysis targeting existing and emerging pathogens, as well as antimicrobial resistance genes and MGEs, revealed that hotspots of AMR transmission were piggery and dairy farms, along with open dumps. These locations served as hotspots for the uptake of extended-spectrum β-lactamases (ESBLs) genes, including blaKPC, and blaNDM, into opportunistic pathogen hosts through horizontal gene transfer. WGS analysis confirmed that among the opportunistic pathogens carrying multiple combinations of ESBLs were Stenotrophomonas maltophilia and E. coli ST410. The latter appeared to be clonal and recently caused outbreaks in Fijian hospitals.

Implications/Applications: Raising awareness and managing the antibiotic resistance crisis is often more challenging in LMICs compared to high-income countries due to limited resources and competing priorities. The proposed genomic surveillance program is beginning to effectively address the AMR crisis by taking into consideration potential constraining factors.

Author: Barbara DRIGO, Future Industries Institute, UniSA STEM, University of South Australia, Mawson Lakes, SA, 5095, Australia

Co-Authors: Djordjevic, Steven P.; Wyrsch, Ethan; Okelo, Walter O.; Magiri, Royford; Lal, Vincent; Donner, Erica

Tuesday, May 28 - 13:45-15:30

Back to the Program

Session 10

Abstract 172

Room

Global distribution pattern, health risks and driving forces of antibiotic resistance genes in livestock manures

The Relative Risk Model, combines data on Antibiotic-Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARG), facilitating the identification of AMR prevalence patterns. It is used in highlighting regional and seasonal hotspots, thereby prioritizing areas for intervention. The Quantitative Microbial Risk Assessment-Disability-Adjusted Life Years (QMRA-DALY) model integrates exposure and health outcomes to estimate the additional health burden attributed to AMR. It serves as a critical tool for quantifying public health impacts and steering effective resource distribution and policy evaluation. Meanwhile, the Antibiotics Risk Model, compares environmental antibiotic levels with established Predicted No-Effect Concentration (PNEC) benchmarks, assessing the risk to aquatic life and broader implications for resistance development. This model informs decisions on antibiotic usage and environmental management, emphasizing ecological preservation.

Author: Xun Qian, Northwest A&F University, China

Co-Authors: Tiedje, James; Li, Bintao; Jiang, Lan; Wang, Guangdong

---

Session 10

Abstract 431

Room 4

Integrative and conjugative elements harboring antimicrobial resistance in bovine respiratory disease and indicator bacteria

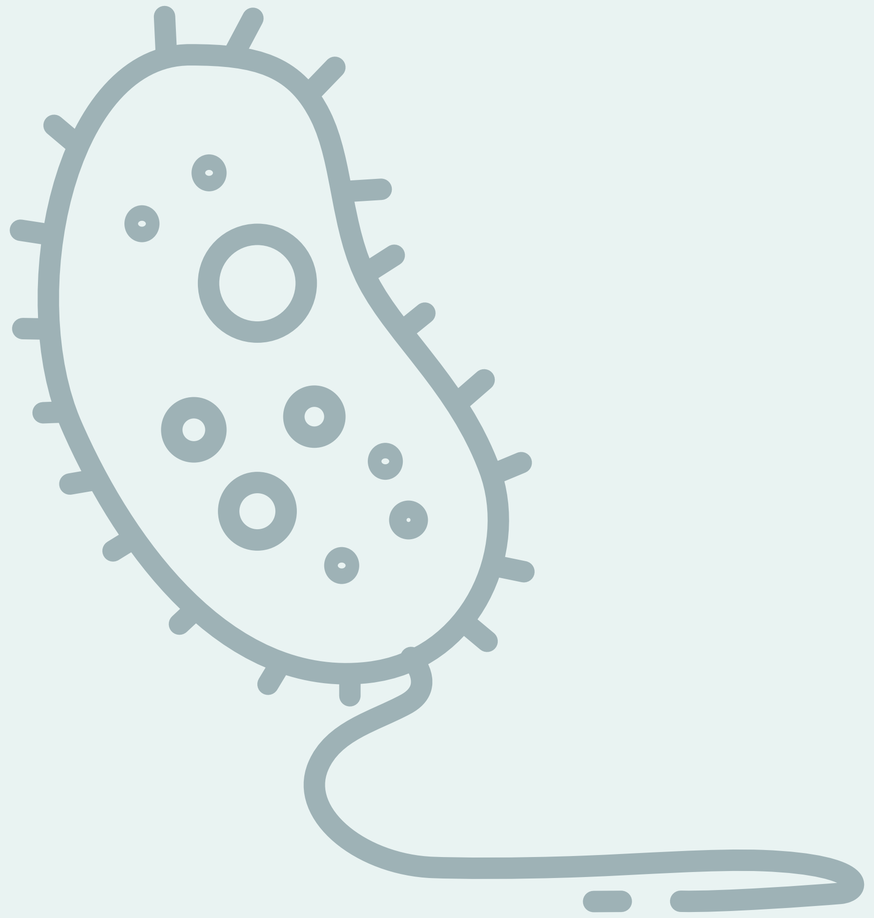
Background and Aim: Integrative and conjugative elements (ICEs) are mobile genetic elements that can transfer autonomously by conjugation in bacteria. ICEs often carry genes encoding various traits, including antimicrobial resistance (AMR). ICE-mediated multidrug-resistance (MDR) in bacterial pathogens of the bovine respiratory disease (BRD) has emerged as a significant cause of AMR, therapeutic failure and mortalities in cattle. This study examined the prevalence and diversity of ICEs and their association with MDR in BRD pathogens and indicator Enterococcus spp.

Method: BRD bacteria including Mannheimia haemolytica, Pasteurella multocida and Histophilus somni, were collected from nasopharynx of beef cattle in North American cow-calf operations and feedlots, and from lung tissue following post-mortem of cattle mortalities. Whole genome sequencing of 463 isolates was conducted using Illumina platform. A One-Health collection of ~1000 Enterococcus spp. genomes from isolates originating from beef cattle production, surrounding environments, municipal waste water, and human clinical samples was also investigated. Genomes were analyzed for the presence of ICEs and AMR genes (ARG). Horizontal transfer of ICEs within and across species was investigated using conjugation.

Results: Among BRD bacteria, over 40 different combinations of ARGs were associated with ICEs, which were integrated at tRNA-leu in the chromosome. Isolates contained up to 14 different ARGs, conferring resistance to up to 7 different antimicrobial classes. Certain ICE modules were conserved across all three BRD bacterial species, suggesting the occurrence of horizontal gene transfer. Conjugation experiments using multi-ARG ICE carrying BRD bacteria as donors demonstrated the transfer of ICEs within and across BRD bacteria, and to human pathogens including E. coli, Haemophilus spp. Klebsiella, and Salmonella. Enterococcus ICEs mostly harboured tetracycline ARGs, while other ARGs were plasmid associated. Implications: This study highlights the widespread nature and ARG diversity of MDR-ICEs within the Pasteurellaceae, and identifies the risk for MDR spread following horizontal transfer. Compared to Pasteurellaceae ICEs, Enterococcus ICEs appear to be of lower risk as they seldom exhibit MDR.

Author: Rahat Zaheer, Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Canada

Co-Authors: Andrés-Lasheras, Sara; Conrad, Cheyenne; Zaidi, Sani-E-Zehra; In Kim, Jee; McAlister, Tim A.



Back to the Program

Session 10

Abstract 20

Room 4

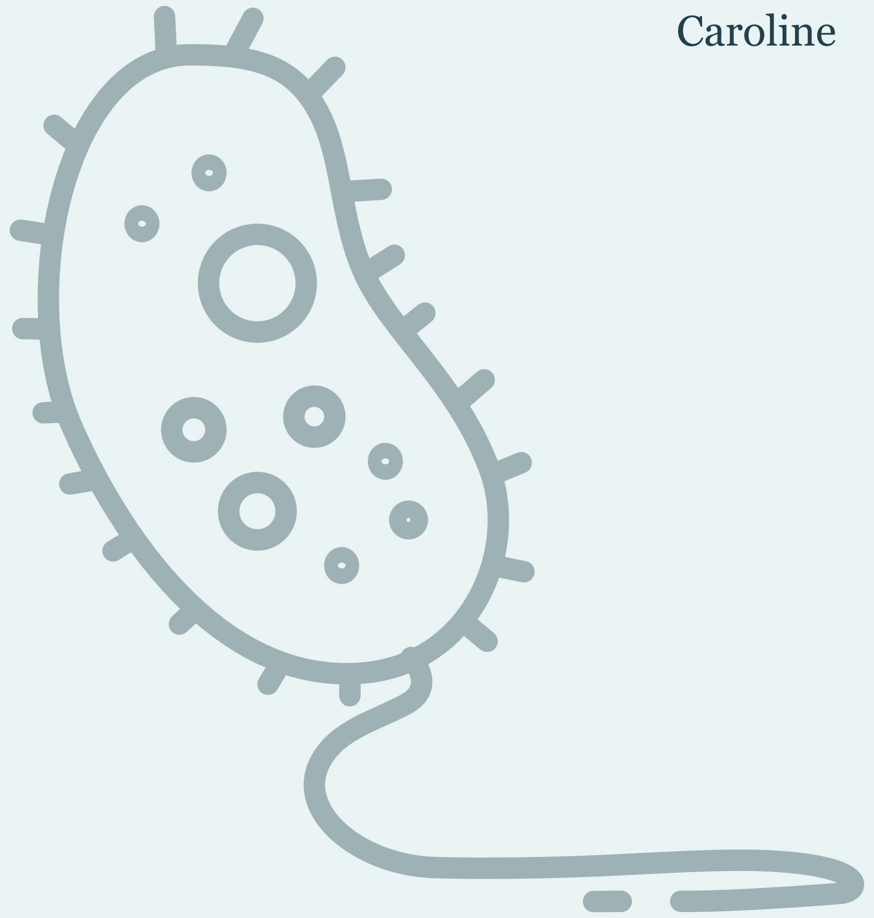
From indoor to outdoor bioaerosols: antibiotic-resistance genes and bacterial diversity from pig finishing buildings and broiler operations in Québec

Background and Aim: Confined animal feeding operations (CAFOs) are important producers of bioaerosols, biological particles suspended in the air. Indoor bioaerosols can be emitted outside the barns through the ventilation system. Bioaerosols can contain antibiotic-resistance genes (ARGs) and contribute to their dissemination in the surrounding communities. This project aims to characterize diversities of ARGs and bacteria emitted from CAFOs in an attempt to better understand the role of air as a vector of antimicrobial resistance (AMR) transmission.

Method: Bioaerosols were collected on electret filters using high volume air samplers. Samples were taken indoor and at 5 different outdoor points depending on the prevailing winds: exhaust fans, 10m, 100 m, 1 km downwind and upwind. A portable meteorological station recorded wind direction and wind speed at each sampling point. Two pig-finishing buildings and two broiler operations were visited three times in the warm season from June 2021 to September 2022. DNA was extracted from filters and used for quantification of 16S rRNA gene and ARGs and amplicon-based sequencing for bacterial diversity. Results. Bacterial concentrations decreased with distance from CAFOs. Aminoglycosides (aac(3), aac-11, aac-1b), beta-lactams (blaOXA, blaIMP, blaTEM), macrolides (erm35, ermT, ermX) quinolones (qepA, qnrB), sulfonamides (sul1, sul2), tetracyclines and vancomycin (vanA, vanB) resistance genes were detected indoor and at the exhaust fans of the four sampled CAFOs. Relative abundances of bacterial genera are similar between the indoor air and exhaust fans too. The bacterial profiles were more dissimilar at the downwind and upwind sampling points. However, the bacterial core remained the same regardless of distance. Implications. ARGs emitted by the exhaust fans can be dispersed over varying distances, exposing surrounding communities and thus contributing to the dissemination of AMR beyond the farms.

Author: Joanie Lemieux, Centre de recherche de l’Institut Universitaire de Cardiologie et de Pneumologie de Québec- Université Laval, Canada

Co-Authors: Dumais, Cindy; Veillette, Marc; Létourneau, Valérie; Turgeon, Nathalie; Duchaine, Caroline



Back to the Program

Session 11

Abstract 423

Room 3

A Standardized Metagenomic Absolute Quantification Method using High-throughput Long-read 3rd Generation Sequencing for Environmental Surveillance of Antibiotic Resistance Genes (ARGs)

The accuracy, read length and throughput of long read 3rd generation sequencing has been significantly improved in the recent years and make it a powerful tool in the study of microbiome. This study aims to improve the long-read metagenomic analysis of absolute abundance of antibiotic resistance genes (ARGs) using a standardized quantification method.

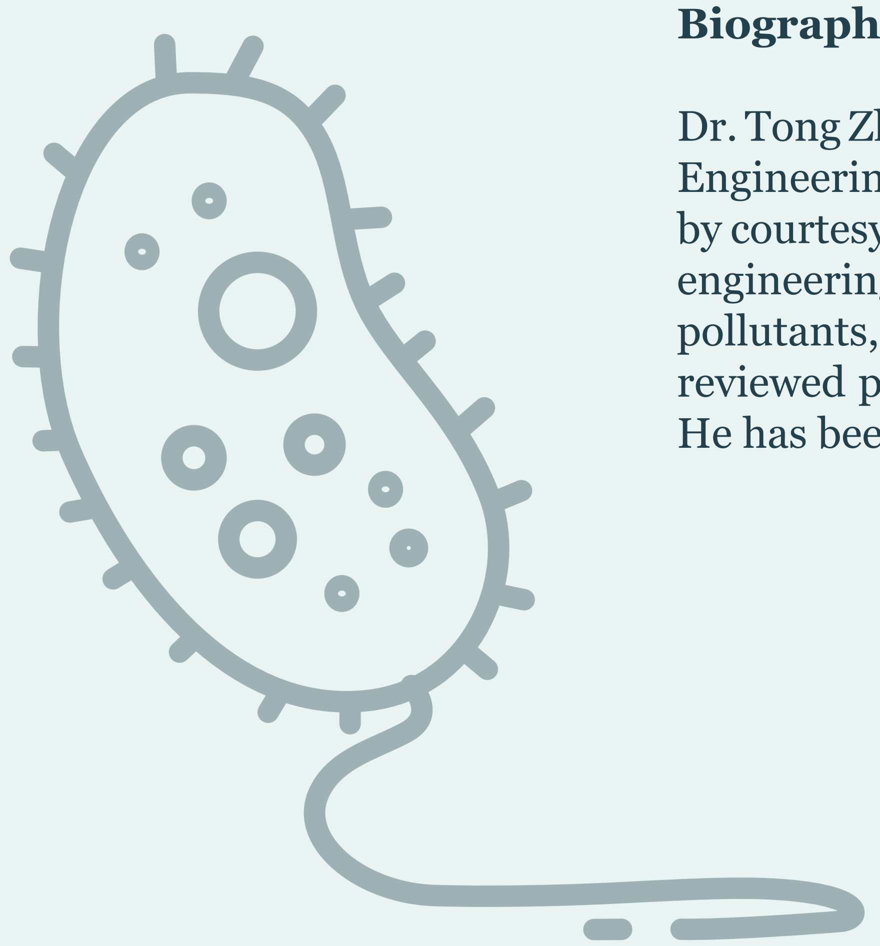
A cellular whole genome spike-in method using two reference species (one G+ and one G-) was developed in this study to correct the DNA extraction efficiency of environmental samples. Additionally, a novel tool called Melon was developed to enhance taxonomic profiling of complex microbial communities using long-read sequencing technology and provide species-level taxonomic abundance. Melon employed a two-stage classification scheme to reduce computational time and included an expectation-maximization-based post-correction module to handle ambiguous reads. By using these methods, the absolute abundances of microorganisms and ARGs in eight types of environmental samples were determined. The relationships between significant microorganisms, ARGs, and mobile genetic elements (MGEs) were explored, and the removal rates of pathogens and ARGs across five wastewater treatment plants (WWTPs) were measured based on absolute quantification results.

The standardized metagenomic absolute quantification method developed in this study is crucial for ARGs analyses, such as profiling, comparison, and host-tracking, and helps address the growing public health threat posed by antimicrobial resistance (AMR). In the future, based on microbial and ARGs profiles obtained using the method developed in this study, risk assessment frameworks will be constructed by integrating factors from quantitative microbial risk assessment (QMRA), ESKAPE-related high-risk ARGs, and faecal indicators to better understand the risks associated with AMR in various environments.

Author: Tong Zhang, University of Hong Kong, Hong Kong

Biography:

Dr. Tong Zhang is a Chair Professor of Environmental Engineering in Environmental Microbiome Engineering and Biotechnology Laboratory at The University of Hong Kong (HKU), and Professor by courtesy in School of Public Health of HKU. His research includes environmental microbiome engineering and biotechnology, environmental bioinformatics, biodegradation of emerging pollutants, antibiotic and antibiotic resistance genes, etc. He has published over 300 peer-reviewed papers, and got more than 40, 000 citations and an H index of 110 (Google Scholar). He has been listed as “Highly Cited Researcher” by Clarivate from 2018 to 2023.



Session 11

Abstract 129

Room 3

A 3D structure and AI-informed search for the origins and dissemination of sulfonamide resistance genes

Background and Aim: Sulfonamide resistance genes (particularly sul1) are surrogate markers of environmental spread and evolution of AMR. They are found on mobile genetic elements (MGE’s), enabling their broad dissemination. Sul enzymes are variants of the sulfonamide drug target FolP enzymes which are essential in the bacterial folate pathway. To date, there are four known plasmid-borne sul genes (sul1 to sul4), however, we do not possess a complete understanding of the size of this family, particularly in the environment and wildlife. We also do not know the origin of these genes. These gaps hinder surveillance and basic understanding of AMR evolution. Here, we undertook a comprehensive computational survey of the Sul family and traced their origins.

Procedure/Method: We leveraged our molecular analysis of the 3D structures of Sul enzymes, plus the Foldseek structure similarity tool, to screen the AlphaFold Structure Database, Genbank, Uniprot and EBI MGnify databases. We used the SSNpipe sequence similarity network tool to cluster thousands of Sul and FolP sequences.

Findings/Results: We previously identified a “resistance motif” in Sul enzymes. Filtering with this, we identified more than 100 new Sul-like enzymes. Their genes are in diverse species and many are chromosomal. Notably, we identified sul-like genes in Actinomycetota, which contain antimicrobial-producing species, localized in operons encoding folate and/or thiamine metabolism genes. This suggests that misuse/overuse of sulfonamides selected for MGE capture and mobilization of housekeeping metabolic genes with sequences enabling intrinsic drug resistance from environmental species.

Implications/Applications:Our findings suggest that the sul family is much larger than previously known, and that they originated in species harboring vitamin metabolism genes enabling intrinsic sulfonamide resistance. These findings can enable broader surveillance and understanding of the evolution and dissemination of AMR. We also demonstrate an effective AI-enabled pipeline for identification of new AMR genes that is generalizable to any family.

Author: Peter Stogios, University of Toronto, Canada

Co-Authors: Venkatesan, Meenakshi; Fruci, Michael; Savchenko, Alexei

Back to the Program

Session 11

Abstract 201

Room 3

ResistoXplorer 2.0: an improved bioinformatics tool for comprehensive statistical, visual and integrative analysis of AMR metagenomic sequencing data

Background and Aim: Antimicrobial resistance (AMR) is considered as one of the most urgent threats to global public health, and metagenomic-based investigations featuring high-throughput analysis of sequence data are increasingly common and important across human, animal, agricultural, and environmental sectors. The resulting large and complex datasets often require powerful, yet user-friendly bioinformatics tools to unveil key patterns, identify significant biomarkers, and explore potential activities.

Method: We previously introduced ResistoXplorer, a user-friendly web-based tool for bench researchers and clinicians to perform comprehensive downstream analysis on AMR genes (resistome) abundance profiles. ResistoXplorer is developed using three open-source programming languages: Java, R, and JavaScript.

Results: Compared to the previous version, ResistoXplorer 2.0 features three new modules: (i) a raw data processing module for metagenomic sequencing data processing and ARGs annotation that connects directly with the ARG Table module for comprehensive visual, statistical and exploratory analysis; (ii) a time series analysis module that incorporates robust, powerful as well as more advanced statistical and visualization approaches tailored for longitudinal studies; and (iii) an interactive 2D/3D network-based visual analytics system providing the topological view of public ARG databases to discover and gain novel biological insights. Other significant improvements include interactive visualizations for common graphical outputs, and updated statistical methods to perform integrative analysis of paired microbiome and resistome datasets. The underlying ResistoXplorerR package will also be released to enable transparent and reproducible analysis.

Applications: ResistoXplorer will be a one-stop shop that allows AMR researchers to thoroughly explore their metagenomic data via intuitive web interfaces. The development of such a bioinformatics tool suite promotes the application of metagenomics-based investigations for surveillance and enhances our understanding of AMR and its dynamics in humans, animals, and environmental microbial communities. ResistoXplorer 2.0 will be publicly available at resistoxplorer.no.

Author: Achal Dhariwal, University of Oslo, Norway

Co-Author: Petersen, Fernanda Cristina

Session 11

Abstract 158

Room 3

ESM_2: A Promising Deep Learning Approach For Identifying Novel Antibiotic Resistance Genes in Environmental Metagenomes

Background and Aim: An aspiration of environmental monitoring is to be able to detect new ARG variants before they become a problem in the clinic. Currently, a “best-hit” approach through comparing DNA sequences derived from genomic or metagenomic data sets to public databases is employed for monitoring ARGs in environmental samples. However, best hit approaches often yield false negatives and can fail to recognize new gene mutations, particularly when they are highly dissimilar from sequences available in public databases. To overcome such limitations, here we introduce the ESM-2 deep learning algorithm, a deep learning model designed to comprehensively identify ARGs in in environmental metagenomes.

Procedure/Methods: HMD-ARG, an extensive manually-curated database consiting of 17,282 sequences with annotated labels for 15 antibiotic classes, 6 resistance mechanisms, and mobility information collated from 7 publicly-available databases, was applied for training the ESM-2 deep learning module. CD-HIT clustering of the HMD-ARG database at 40% similarity partitioned samples for training (80%) and unique test set evaluation (20%). BLAST classified test ARGs by sequence homology against HMD-ARG database. The ESM-2 neural network independently categorized test cases among antibiotic classes after training on the larger dataset.

Findings/Results: Compared to BLAST’s 23% test accuracy, constrained by lack of homologs for many ARGs, ESM-2 achieved 71% accuracy in categorizing ARGs spanning all antibiotic classes. Across precision and recall metrics, 3-fold enhancements demonstrated ESM-2’s strengthened capacity to capture a diverse range of novel ARGs that are missed via best-hit approach

Implications/Applications: ESM-2 shows promise for discovering emerging ARGs in environmental metagenomes. By classifying ARGs directly from metagenomic sequencing reads, this tool could enable more scalable and reliable monitoring that captures evolution of ARGs across diverse ecological niches.

Author: Vineeth Manthapuri, Virginia Polytechnic Institute and State University, United States

Co-Authors: Dasu, Pradyumna Upendra; Shukla, Anmol; Ahmed, shafayat; Wang, Xuan; Zhang, Liqing; Pruden, amy

Back to the Program

Session 11

Abstract 285

Room 3

Harmonizing/integrating surveillance data in the One Health continuum: The GRDI One Health AMR pathogen genomic surveillance contextual data standard

Background and Aim: One Health initiatives often involve heterogeneous data streams originating from different sources/agencies/sectors/information management systems posing challenges for data harmonization, integration and meaningful interpretation. Data standards enable this information to be more easily reused for different types of analyses. The federal Canadian Genomics Research and Development Initiative for AMR (GRDI-AMR) uses a genomics-based approach to understand how environmental determinants and food production contribute to the development of AMR of human health concern. To better harmonize GRDI-AMR pathogen genomics contextual data across data providers, an ontology-based data specification was developed, tested and implemented by partners across the One Health continuum.

Procedure/Method: A data needs assessment identified needed data elements for analysis. Vocabulary was standardized according to best practices and existing standards/ontologies were reused where possible. Data was structured using a framework developed by the authors for ISO 23418. The specification was tested through a series of data curation pilots involving curators from different agencies. Feedback was incorporated into the standard. The standard was then mapped to other existing organizational-specific specifications.

Findings/Results: The specification enabled the harmonized capture of contextual data pertaining to sample collection, host/strain/isolate information, AMR profiles, sequencing, and risk assessment data. The specification was implemented via a modular, spreadsheet-based collection template as well as curation app used nationally during the pandemic (the DataHarmonizer), and accompanied by a variety of support material. Mapping to downstream applications enabled tooling for automated data transformations for sequence submissions. The specification package is version controlled and available at https://github.com/cidgoh/GRDI_AMR_One_Health.

Implications/Applications: The specification is currently being implemented across GRDI-AMR partners, with a focus on a comprehensive integration within the federal genomics ecosystem. While designed for Canada, the data standard is also readily adaptable for international use.

Author: Emma Griffiths, Simon Fraser University, Canada

Co-Authors: Shay, Julie; Sehar, Anoosha; Cameron, Rhiannon; Barclay, Charlotte; John, Nithu Sara; Scott, Andrew; Wajnberg, Gabriel; Robertson, James; Schonfeld, Justin; Bastedo, Patrick; Jurga, Emil; Johnson, Lisa; Kess, Tony; Nash, John; Topp, Ed; Taboada, Eduardo; Van Domselaar, Gary; Smith, Derek; Broadbent, Jordyn; Poulin-Laprade, Dominic; Laing, Chad; Zaheer, Rahat; Reid-Smith, Richard; Carrillo, Catherine; Hsiao, William

Tuesday, May 28 - 16:00-17:00

Back to the
Program

Session 12
Abstract 381
Room 4

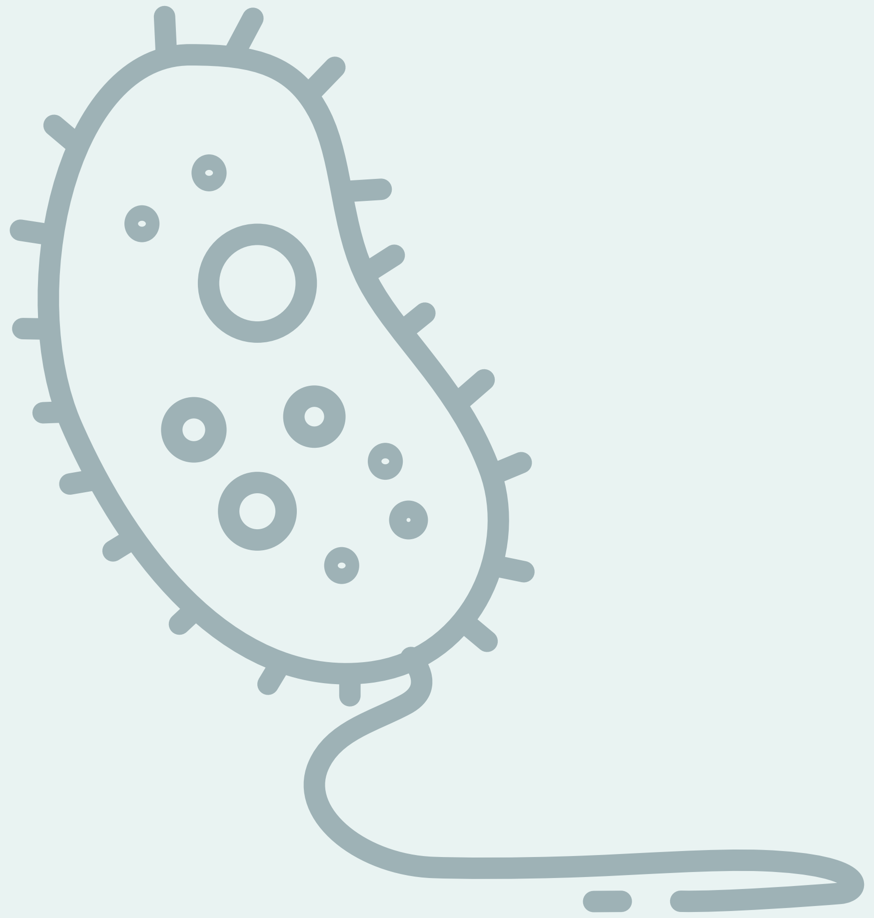
Production of antimicrobials by Canadian High Arctic bacteria

With a global antibiotic resistance crisis and a dry research and development pipeline for new and effective antibiotics, researchers are exploring new avenues for identifying novel antibacterial compounds. We propose to investigate bacterial isolates from the Canadian High Arctic, an extreme environment with high microbial diversity and unique environmental pressures. The goal of this project is to determine if Arctic isolates produce secreted compounds that could represent novel antibiotics. Screening of Arctic isolates from our collaborators in the Whyte lab led to the identification of six bacterial strains with antibacterial activity against several clinically relevant pathogens. We then characterized the antibacterial activity of organic extracts from these isolates against relevant clinical pathogens after confirming activity against the hyper-permeable and efflux deficient E. coli ΔbamB ΔtolC. We developed a high-throughput bioluminescence assay using E. coli ΔbamBΔtolC to identify antibiotic activity from culture supernatants and used this assay to optimize antibiotic production of our candidate isolate. Following fractionation of our candidate extract using reiterative rounds of High-Performance Liquid Chromatography coupled to Mass Spectrometry we have isolated a candidate bioactive compound. We will determine the potency and spectrum of activity of this compound against relevant clinical pathogens. The structure of antibacterial compound(s) will then be elucidated using Nuclear Magnetic Resonance. This work will highlight the value of the Arctic bacterial isolates as a source for novel antimicrobials.

Author: Eszter Farkas, McGill University, Canada

Biography:

Eszter completed her BSc in Translational and Molecular Medicine in 2020. She then began her Master's at McGill University under the supervision of Dao Nguyen and co-supervised by Samantha Gruenheid. She then fast-tracked to the PhD program in 2022 and she is currently a PhD candidate studying the production of antibiotics by Canadian High Arctic bacteria. Besides her graduate studies, she is involved in the McGill AMR Centre Outreach team and the Scientista mentorship program.



Session 12
Abstract 35
Room 4

Biosynthetic Potential of Antibacterial Compounds Determines Soil Antibiotic Resistomes of Different Land-Use Patterns

Background and Aim: Soil is recognized as the major reservoir of antibiotic resistance genes (ARGs). However, the health risks of soil ARGs and the driving factors for their distribution in different land-use patterns remain poorly understood. In this study, we aim to profile the high-risk ARGs and investigate the predominant factors in shaping soil resistomes in cropland and forest soils.

Procedure/Method: we conducted a nationwide metagenomic investigation of 70 cropland and 39 forest soils in China and used experimental datasets of manure-application to validate the main results.

Findings/Results: The richness and abundance of high-risk ARGs (the mobilizable and pathogen-carried ones) in cropland soils (4.3 subtypes, 1.4×10-3 Gene copies Per Cell) was 8.4 and 25.7 times higher than that in forest soils (0.51 subtypes, 5.6×10-5 GPC), respectively. The results indicate the increased risk level of soil resistomes by anthropogenic activities. However, contribution analyses showed that the microbial biosynthetic potential of antibacterial compounds, rather than anthropogenic activities or environmental factors, was the most important determinant in shaping both cropland and forest soil resistomes. The abundance of biosynthetic gene clusters (BGCs) producing antibacterial compounds was significantly and positively correlated with the abundance of ARGs in cropland and forest soils. Further analyses untangled that both self-resistance of producers and antagonistic interactions within microbes contributed to the ARG abundance, of which self-resistance ARGs account for 14.1%-35.1% of total ARGs. Notably, we observed the significantly positive correlation between antibacterial BGCs and high-risk ARGs in manure-applied experiments, indicating that natural antibacterial compounds could promote the dissemination of high-risk ARGs.

Implications/Applications: These findings reveal the dominant role of natural antibacterial compounds on the emergence and dissemination of soil ARGs and provide critical implications on the risk control of soil resistomes, for example, the careful application of livestock manures (usually carrying abundant high-risk ARGs).

Author: Zhiguo Zhang, Westlake University, China

Co-Author: Ju, Feng

Tuesday, May 28 - 16:00-17:00

Back to the Program

Session 12

Abstract 196

Room 4

Development of control technologies of antibiotics and environmental antibiotic resistance dissemination in pharmaceutical industry

China is the world's largest producer of active pharmaceutical ingredient (API) for fermentative antibiotics. High concentration of antibiotics in antibiotic production wastewater inhibits the traditional biological wastewater treatment system and enhances the antimicrobial resistance (AMR) development. Hence, the selective elimination of antibiotic pressure before antibiotic production wastewater entering the biological treatment system is the best way to simultaneously control conventional contaminants, antibiotics and antibiotic resistance genes (ARGs).

Enlightening by the easy-to-hydrolyze property of fermentative antibiotics, we developed a novel pretreatment technology based on enhanced hydrolysis by using homogeneous or heterogeneous solid acid/base catalysts for targeted elimination of antibiotic potencies from wastewater. A prediction model of enhanced hydrolysis efficacy was constructed, and a classification treatment strategy for antibiotic wastewater based on antibiotic removal and AMR transmission blocking was proposed. Enhanced hydrolysis pretreatment together with UASB reactor has been successfully applied to the treatment of oxytetracycline manufacturing wastewater in two sites in Hebei, China (800 m3/d and 1,000 m3/d, respectively). The abundance of ARGs in biological treatment units could be reduced by over 83%, and the challenge on biological inhibition was also solved. This work has contributed to the WHO Technical Brief on Water, Sanitation, Hygiene and Wastewater Management to Prevent Infections and Reduce the Spread of Antimicrobial Resistance, the Blue Book of China's Pharmaceutical Industry, and the China Pharmaceutical Industry EHS Guideline, which will provide scientific guidance for AMR management in global pharmaceutical industry.

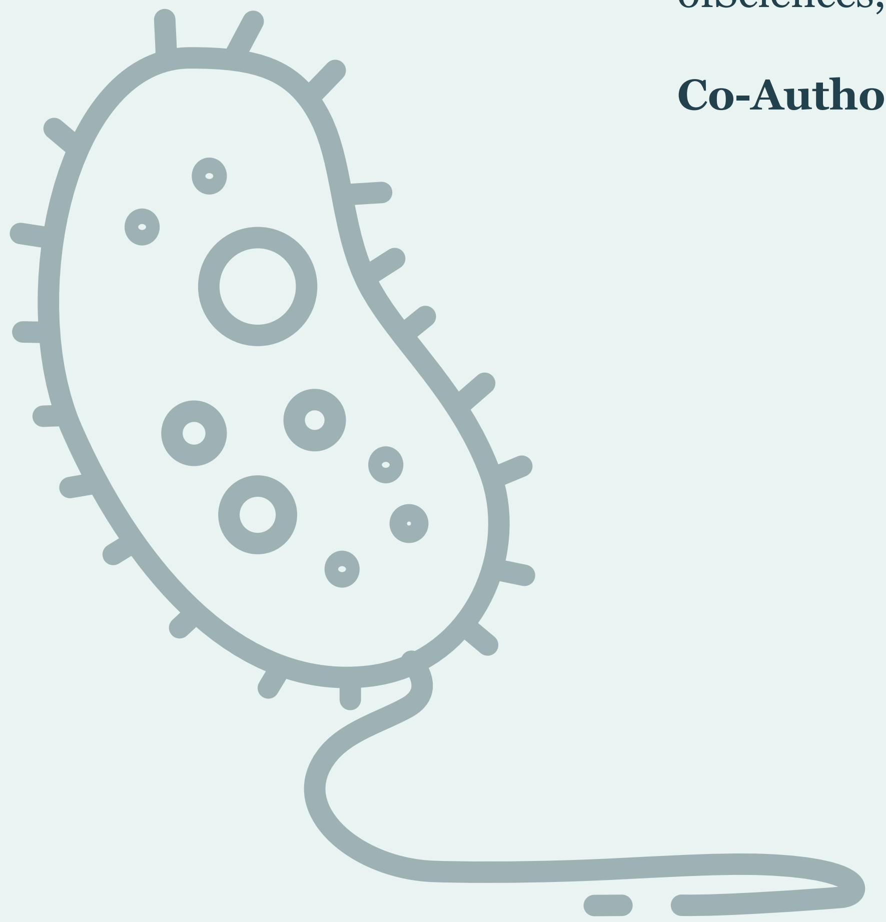
To decrease the negative impact of denatured protein particles on anaerobic biological treatment, filtration step after enhanced hydrolysis pretreatment is useful for improving treatment efficiency. In the future, anaerobic membrane bioreactor (AnMBR) is promising in treating antibiotic production wastewater with short process flow and low AMR effluent.

Acknowledgement

This work was supported by National Natural Science Foundation of China (Grants 32141002).

Author: Min Yang, Research Center for Eco-Environmental Sciences, Chinese Academy ofSciences, China

Co-Authors: Wang, Chen; Feng, Haodi; Han, Ziming; Liu, Shihai; Dai, Shiting; Zhang, Yu



Tuesday, May 28 - 16:00-17:00

Back to the Program

Session 13

Abstract 422

Room 3

Population genomic approaches to unravelling evolution of drug resistance in pathogenic fungi

Over recent decades we have seen a rise in the number of infections and outbreaks caused by pathogenic fungi in humans, plants and animals. It is a matter of concern that a rising proportion of these infections and outbreaks are resistant to antifungal drugs. Population genomics provides a framework to assess the evolution of resistance and its subsequent spread, and in this talk we discuss how these approaches have been successfully applied to critical priority fungal pathogens.

Author: Johanna Rhodes, Imperial College London, United Kingdom

Biography:

After completing her PhD in host gene regulatory networks activated in response to fungal infection at the University of Warwick, Jo moved to Imperial College London to research the pathogen itself, and focus on human infection. Her research has focused on three of the four WHO Critical Priority Group fungal pathogens: Cryptococcus neoformans, Candida auris and Aspergillus fumigatus. Now at Radboudumc in the Netherlands as a PI, Jo's research group uses a One Health approach to balance and optimise the health of humans, animals and the environment.

Session 13

Abstract 128

Room 3

Citizen scientist surveillance of azole-resistant Aspergillus 12 in UK airs and soils

Background and Aim: Azole-resistant Aspergillus fumigatus (ARAf) is truly a one-health problem. This mould plays an important role in the environment as a decomposer but is also an opportunistic human lung pathogen if the inhaled spores are not cleared by our innate immune system. A. fumigatus spores in the environment can develop resistance to azole compounds when they are exposed to agricultural pesticides, which results in aspergillosis lung infections that are resistant to the azole drugs used to treat them. The aim of this study was to establish the proportion of environmental A. fumigatus spores in the UK that are azole-resistant.

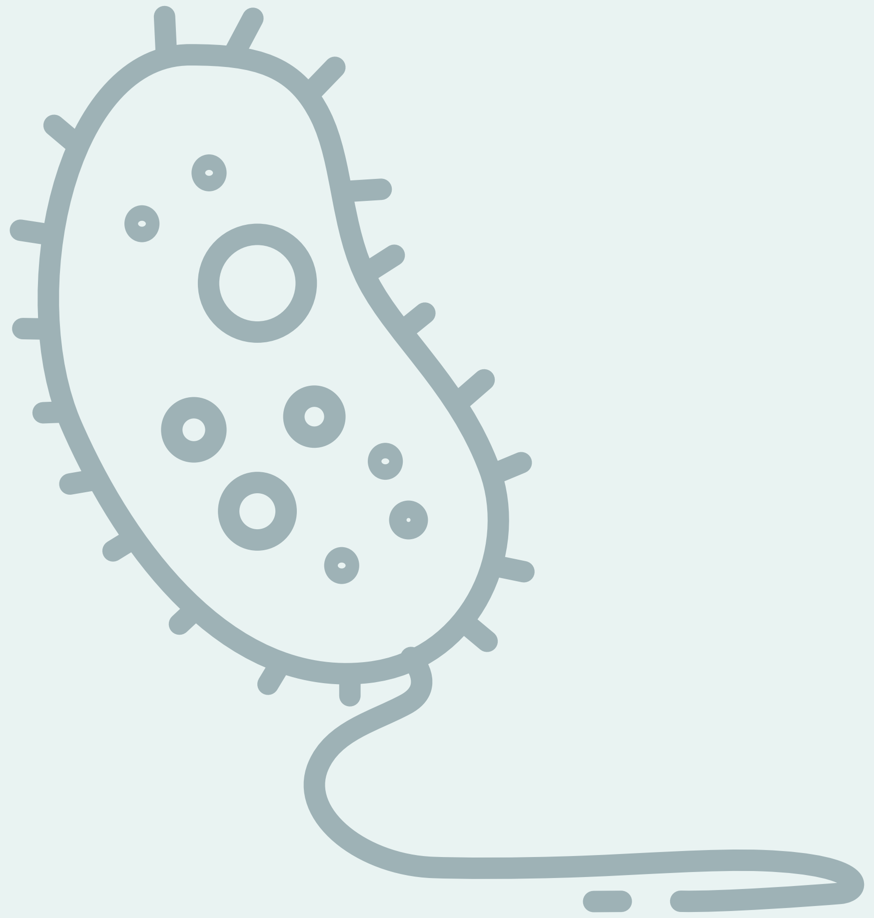
Method: Between March 2018 and March 2019, two citizen science campaigns "Science Solstice" and "Summer Soilstice" asked volunteers to collect air and soil samples from their homes and gardens. These samples were sent to the UK Centre for Ecology & Hydrology (UKCEH) where they were cultured for A. fumigatus spores, which were tested for susceptibility to a panel of azoles.

Results: Across both campaigns, 787 citizen scientists collected 1,894 air samples and 506 soil samples, from which were cultured nearly 8,000 A. fumigatus isolates. The prevalence of azole-resistance in the airborne spores was 5% and in soilborne spores was 14%.

Applications: The success of these citizen science campaigns for ARAf surveillance has lead to the LatAsp survey involving citizen scientists in 12 countries across Latin America. Our follow-on project MARAf (Monitoring for ARAf) is a citizen science project involving homeowners, farmers and industrial composters across the UK in both air and soil sampling, to establish where azole-resistance is developing. This project is being run in collaboration with The Netherlands, who ran a nationwide citizen science surveillance campaign in November 2023, with all methods being kept standardised such that results can be compared between the UK and The Netherlands.

Author: Jennifer Shelton, UK Centre for Ecology and Hydrology, United Kingdom

Co-Authors: Stanton, Isobel; Payne, Rachel; Rhodes, Johanna; Uzzell, Christopher; Hemmings, Samuel; Brackin, Amelie; Sewell, Tom; Dyer, Paul; Alghamdi, Asmaa; Borman, Andrew; Johnson, Elizabeth; Piel, Frederic; Singer, Andrew; Fisher, Matthew



Wednesday, May 29 - 8:30-9:30

Back to the Program

Session 14

Abstract 433

Room

What does integration look like? - One Health-thinking, harmonised methods, and global context

Background and Aim: Antimicrobial resistance (AMR) poses a significant global health challenge. Projections suggest that over 10 million deaths will occur annually by 2050 from AMR-related causes, with particularly severe economic and social impacts in low-income countries. Until recently, solutions to AMR have centred on the human and animal health sectors; however, the mediating role of environmental, plant, and foodborne pathways in AMR development, transmission, and spread is now apparent.

Methods: Addressing global AMR requires a 'One Health' approach that recognizes the interconnectedness of human, animal, plant, and environmental health. The recent United Nations report, ""Bracing for Superbugs,"" suggests that One Health-based solutions are needed, emphasizing that AMR prevention should be our first goal in conjunction with integrated global AMR surveillance to help prioritize interventions. Integration here means the harmonization of terminologies, methods, and data across sectors and regions, as well as an expansion of basic capacities across all countries.

Results and Implications: This presentation will first examine “integration” in terms of the needs of a surveillance network. It will then use three case studies to illustrate what “effective” integrated AMR surveillance might entail. The cases include: 1) characterisation of neighbourhood-level AMR transmission among Bangladeshi infants, 2) city-level AMR levels in wastewater to assess AMR prevalence and diversity in the UK, and 3) the use of Artificial Intelligence methods to identify regional, sectoral, and methodological knowledge gaps that must be resolved to enable a global-level AMR surveillance capacity. The talk will especially emphasize the importance of semantic and methodological harmonization across regions and sectors as key to creating a truly integrated AMR surveillance network.

Author: David W Graham, Newcastle University, United Kingdom

Biography:

David Graham has led AMR and other surveillance projects around the world for 30 years. He co-led writing the 2023 UNEP report “Bracing for Superbugs: Strengthening Environmental Action in the One Health Response to Antimicrobial Resistance” and chairs integration of the upcoming “Quadripartite Guide on One Health Integrated Surveillance of Antimicrobial Use and Resistance”. He co-initiated the Pan-American Network for Environmental Epidemiology (PANACEA) and co-authored the 2021 World Economic Forum report on the economics of waterborne AMR. Domestically, he was a member of the Strategic Advisory Group for Emergencies sub-group on COVID-19 Transmission in the Environment and the Expert Advisory Group who operationalised the UK wastewater surveillance network during the pandemic. He is a Chartered Professional Civil Engineer with expertise in microbial genetics and environmental chemistry, providing him a broad background suited to integrated AMR studies – from hydrodynamics to metagenomics. His current interests span the ecology of AMR gene exchange to identifying research and communication gaps that hinder One Health solutions.

Session 14

Abstract 80

Room 3-4

One Health, One Data: Describing Antimicrobial Resistance in Surface Water in the United States

Background: The National Antimicrobial Resistance Monitoring System (NARMS) has monitored antimicrobial resistance associated with pathogens of humans and animals since 1996. To implement a One Health strategy, a pilot was initiated to assess antimicrobial resistance across the environment by evaluation of surface water.

Methods: Multiple government agencies are collaborating to evaluate water collection strategies, laboratory methods, and analysis and reporting approaches. Data from a focused watershed study is being contrasted to data from hot spot analysis (ie; high human, high agricultural, and high nature impact). Additionally, metagenomic data is being contrasted to quasimetagenomic data (shotgun sequencing of enrichments) to assess which data type will provide the most relevant information for the monitoring of clinically important antimicrobial resistance. Additional foci of the monitoring effort include description of bacterial species most highly correlated with global AMR mortality, as well as Veterinary Laboratory Investigation and Response Network (Vet-LIRN) and European Antimicrobial Resistance Surveillance network of veterinary medicine (EARS-Vet) monitoring targets.

Results: Interestingly, β-lactam resistance genes were prevalent in twice as many of the high developed sites compared to natural sites. Across specific samples, the AMR detected by enriched (quasimetagenomic) data described 30% of NARMS surveillance targets contrasted to 1% observed by metagenomic data. Quasimetagenomic data also described more plasmids than culture independent metagenomic data. Certain pathogens, however, could only be detected in metagenomic data.

Implications: Data from these initiatives will serve as a baseline for future monitoring work across the country and internationally. Samples from ‘high nature’ may infer an intrinsic component of resistance that will be useful to contrast against anthropogenic selective pressures. Certain laboratory methods and informatic approaches were shown to be more useful for specific aims. These data will be used to inform antimicrobial policies for medical, agricultural, and food production practices.

Author: Andrea Ottesen, FDA, United States

Co-Authors: Kocurek, Brandon; McDermott, Patrick; Strain, Errol

Wednesday, May 29 - 8:30-9:30

Back to the Program

Session 14

Abstract 160

Room 3-4

Evidence-Integrated Insights on Antimicrobial Resistance for better understanding "One-Health"

Background and Aim: Antimicrobial resistance (AMR) is a growing One Health problem, which poses a multisectoral threat to human, animal, food supply, environment, and socioeconomic. However, the understanding of AMR from a One Health approach is limited. Through AI-based methods and complex network analysis, we aimed to provide evidence on overlaps between sectors and identify future opportunities for AMR research and solutions from a One Health perspective.

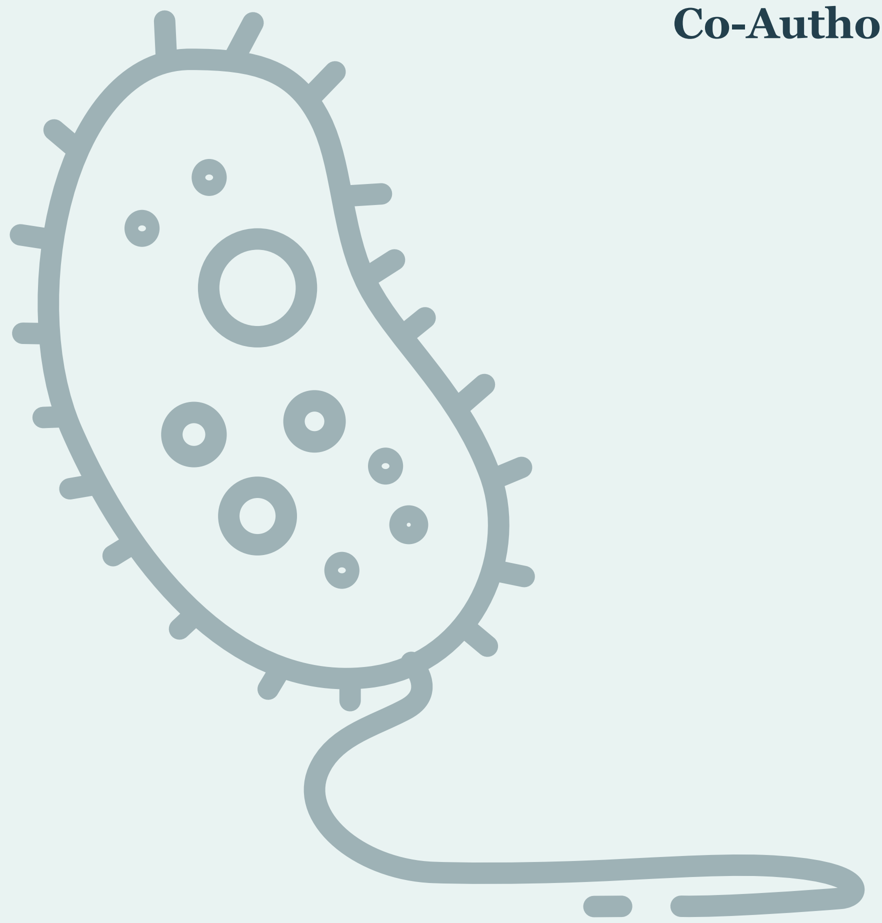
Procedure/Method: We identified 140,973 AMR research related to One Health from 2003 to 2023. We first established a broad semantic list related to sectors of human health, plant health, animal health, environment, and food systems based on the new One Health Joint Plan of Action (2022–2026). We utilized Natural Language Processing (NLP) to identify sector-related literature and determined the development stages of AMR research by hierarchical clustering. Complex networks were designed to identify intrasectoral and intersectoral AMR research.

Findings/Results: We identified three development stages of AMR research: 2003-2011, 2012-2018, and 2019-2022. The number of inter-sector research increased by 3.5 times over the period assessed, showing growing associations between sectors. The overlap of research was primarily at the human health and environment interface. Overlapping research between the environment and plant health sectors increased most sharply from stage 1 to stage 3. Conversely, the amount of plant health research was limited relative to other sectors. More than 40% of multisectoral research was conducted in high-income countries (HICs). Unfortunately, only 33 publications contained work in all five sectors, and none were from low-income countries (LICs).

Implications/Applications: This analysis is the first to utilize NLP-based methodology to integrate knowledge from AMR research and explore the multisectoral relationships under One Health approach. We constructed a data-driven framework of numerous, cross-disciplinarity, and cross-sector studies, which provided evidence for guiding the prioritising work to combat AMR under One Health approach.

Author: Cai Chen, Institute of Urban Environment, Chinese Academy of Sciences, China

Co-Authors: Li, Shule; Zhu, Yong-guan; Graham, David



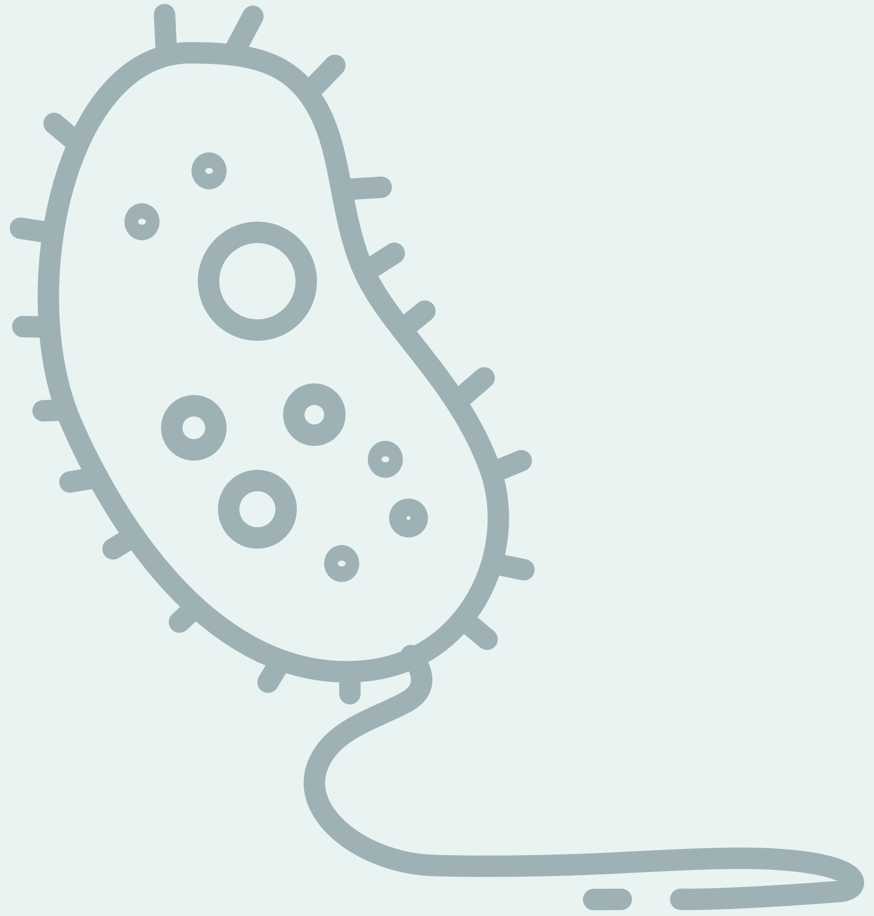
Back to the Program

Session 15: Technical Exhibitor Session

Resistomap Intelligece Platform
for environmental AMR monitoring
Windi Muziasari - Resistomap

Minimizing Bias in
Microbial Research
Bylan Barbera - Qiagen Inc

Illumina's XLEAP Chemistry
Unlocks Accessibility to Highly
Sensitive AMR Detection
Jeffrey Gross - Illumina ULC



Workshop 1 - EDAR in Asia-Pacific LMICs – Research needs and Regional Guideline for AMR surveillance in the Animal Farm Environment

Asia-Pacific LMICs are considered a critical area for global spread of AMR due to dense population and rapid development. There is an urgent need for an environmental AMR monitoring framework, identifying key sources and hotspots for effective management in the One Health approach. However, AMR's fate and spread under local conditions of pollution and climate remains poorly understood. The Part-I of workshop aims to collectively identify research needs for a globally integrated environmental AMR monitoring framework suitable for the Asia-Pacific LMICs.

FAO Regional Office for Asia and the Pacific has drafted a Guideline on AMR Surveillance in Animal Farm Environments, as part of the regional guideline series on surveillance of AMR, AMU and antimicrobial residues. This designed to be aligned with existing standards, anchored in regional context, and in consultation with countries and experts on this theme. The insights of environmental practitioners at EDAR7 on this draft are sought as part of the consultation process, during Part II of this workshop.

This workshop is organized by:

Prof. Ryo Honda (Faculty of Geosciences and Civil Engineering, Kanazawa University, Japan)
Dr Muhammad Usman Zaheer (Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific, Tailand)

Workshop 2 - Strengthening the Policy Relevance of Wastewater-based Surveillance for AMR

The major objective of the workshop is to inform and advance policy relevance of antimicrobial resistance (AMR) measurements generated by wastewater-based surveillance (WBS) programs. Wastewater offers substantial promises to complement and bolster current AMR surveillance programs, which overwhelmingly rely on data collected within clinical settings. However, the extent to which wastewater data provides actionable or policy relevant insights remains unclear. The goal of this workshop is to bridge the gap between data collection strategies and actionable policy. The workshop will integrate discussion with breakout groups to address:

1) What current public health relevant indicators are used to drive antimicrobial stewardship policy at local/global/national scales?

2) What public health relevant indicators can be derived from wastewater through monitoring programs?

3) What information, resources, and contextualization is/are needed to align public health indicators derived from wastewater with other public health indicators to better inform epidemiological understanding of AMR?

4) How can the information derived from WBS and wastewater-based epidemiology (WBE) contribute to the formulation of effective public health policies or interventions?

This workshop is organized by:

Mrs. Sheena Conforti (Ph.D. Student, Eawag, Swiss Federal Institute of Aquatic Science and Technology, Switzerland)
Prof. Amy Pruden (Virginia Tech, USA)

Workshop 3 - Exploring the Factors Influencing Antibiotic Resistance Beyond the Wastewater Pipeline

The workshop focuses on how complex ecological processes occurring in effluent-environmental interfaces (receiving water, soil, crops) influence the mitigation/persistence of antibiotic resistant bacteria and antibiotic resistance genes (ARB&ARGs). Mitigation/persistence of effluent-derived ARB&ARGs depend on processes taking place within these interfaces that are strongly influenced by environmental conditions, and therefore, a holistic understanding of these processes necessitates synthesis of ecological, epidemiological and environmental approaches. Eutrophication, pollution or water reuse, are likely to increase the contamination by ARB&ARGs. However, no single factor can be claimed to be responsible for the spread or containment of AR in the receiving environment.

In this workshop, our objective is to comprehensively examine the diverse array of variables and factors influencing the enhancement or mitigation of antibiotic resistance upon treated wastewater release into the environment. We aim to foster participant contributions towards identifying specific biotic and abiotic factors that control the fate of ARB&ARGs in wastewater-receiving environment and explore how these factors can be managed to mitigate ARB&ARG in the complexity of environmental settings.

This workshop is organized by:

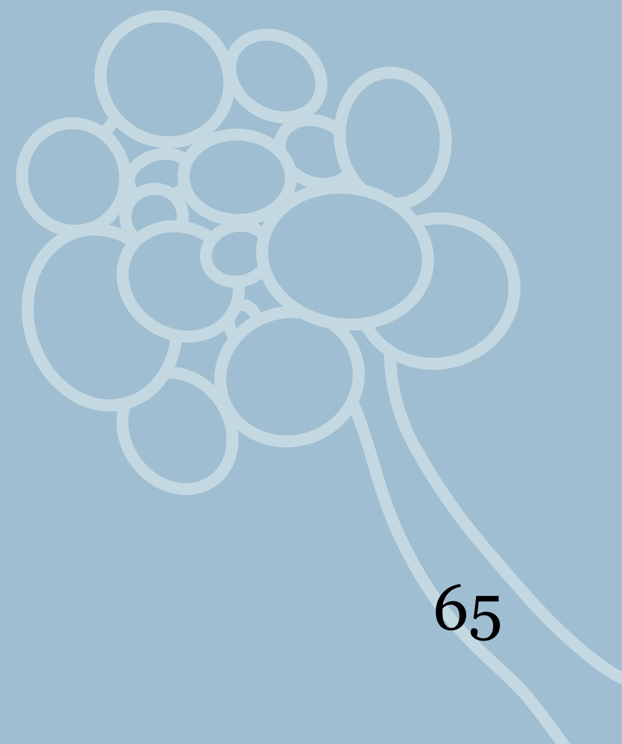
Prof. Célia M. Manaia (Universidade Católica Portuguesa - Porto, Portugal)
Prof. Despo Fatta-Kassinos (University of Cyprus, Cyprus)
Prof. Thomas U. Berendonk (TU Dresden, Germany)
Prof. Christophe Merlin (CNRS/University of Lorraine, France)
Dr. Eddie Cytryn (Volcani Institute, Agricultural Research Organization, Israel)

Workshop 4 - Mutual understanding between regulatory needs and science-based risk assessment of environmental AMR

The aim of understanding the environmental dimension of antimicrobial resistance is to deduce mitigation measures from this knowledge. Such mitigation measures may be technological interventions (e.g. special ways of wastewater treatment) or regulatory measures. Regulatory measures resulting in technical interventions or restrictions in the use of antibiotics require knowledge on the presence of particular hazards and exposure routes to the hazards as well as risk assessment schemes that allow to reach tractable decisions. In addition, risks assessment schemes rely on (standardized) methods to provide data for the assessment. Moreover, ideally the impact of an intervention or restriction is measurable to confirm its efficiency and to weigh their costs against the benefits. The aim of the workshop is to create a mutual understanding between regulators, industry and scientists of the regulatory frameworks currently in place and explore how the available science-based risk assessment schemes and methods can help to reach regulatory decisions.

This workshop is organized by:

Prof. Dr. Thomas Heberer (Federal Office of Consumer Protection and Food Safety and Technical University of Berlin, Germany)
Dr. Svenja E. Sander (Federal Office of Consumer Protection and Food Safety, Germany)
Dr. Frank Schreiber (Federal Institute for Materials Research and Testing, Berlin, Germany)
Dr. Birgit Walther (German Environment Agency, Berlin, Germany)



Workshop 5 - The PACCARB Experience: Integrating the Environment into AMR Policy

Armed with crucial insights from PACCARB reports like “Preparing for the Next Pandemic,” “Bridging the Gap: Improving Antimicrobial Access and Use Across One Health,” this interactive and audience-engaged workshop tackles the critical challenge of integrating environmental action into national policies and will provide insight into the PACCARB’s development as an advisory council grounded in a One Heath-based approach. We will dissect previous PACCARB environmental recommendations and delve into the anticipated “Combatting Global AMR” report due in May 2024, analyzing its potential impact on national action plans. Expert panelists representing diverse disciplines will discuss the challenges in policy discussions and approaching multidisciplinary topics and perspectives with the goal of highlighting the intricate connections of One Health, particularly the environment, to AMR-related policies. Participants will gain a comprehensive understanding of the policy development process in the US, best practices on convening subject matter experts from across a range of subjects with the goal of highlighting the complexity of AMR to a range of audiences, and the dimensions of AMR policies recommended by the PACCARB to date.

This workshop is organized by:

Presidential Advisory Council on Combating Antibiotic-Resistant Bacteria (PACCARB; Affiliation: US Department of Health and Human Services)
Dr. Jomana Musmar (Presidential Advisory Council on Combating Antibiotic Resistant Bacteria, USA)
Dr. Zanah Francis (Presidential Advisory Council on Combating Antibiotic Resistant Bacteria, USA)

Workshop 6 - Gaming AMR: Role-Play Simulation Exploring the Policy Dimensions of Antimicrobial Resistance

Participants will be introduced to the policy process, the role of stakeholders in policy deliberations, and how scientific and technical information is used in such deliberations through a role-play simulation exercise. Participants will take on the roles of different stakeholders relevant to the AMR issue and deliberate around a framework for the development of a National Action Plan to combat antimicrobial resistance. Participants will discuss several different dimensions of AMR, including antibiotic production, wastewater surveillance, growth promotion in agriculture, the prescribing of antibiotics, and the education of healthcare workers. The importance of science in decision-making and the influence of stakeholders' interests, values, and priorities in negotiations will be explored. The exercise will be followed by a debrief conversation to allow participants to talk about what happened during other games and give them a chance to talk about the connection of policy with their day-to-day work.

This workshop is organized by:

Mrs. Rebekah Riddle (PhD student, Science-Policy Interface (SPI) Lab, Virginia Tech, USA)
Prof. Todd Schenk (School of Public and International Affairs, Virginia Tech, USA)

Workshop 7 - New detection methods for AMR in managed agriculture and environment

Although AMR has attracted increasing attention, the mechanisms for AMR spread among bacteria, fungi, parasites and viruses between sources is very complex and poorly understood. Certain routes of AMR transmission can occur between the environment, humans, and animals. Managed agricultural environments may be key sources of AMR, impacting receiving environments, livestock and humans through a variety of pathways.

This workshop aims to tackle these challenges by taking a transdisciplinary approach to understand the complexity and diversity of AMR sources, pathways, and receptors in managed agricultural environments, including biosensing, microfluidics, molecular method and big data, associated with a UK-Canada BBSRC funded project involved world-leading universities and institutes from both the UK and Canada as well as from other countries.

It will bring together inter disciplinary scientists to discuss and brain storm on AMR challenges being faced in Canada and UK and draw synergies and bespoke approaches and align this to proposed techniques such as biosensing, microfluidics, and point-of-use sensors. These will inform the development, and prioritization of interventions, to reduce environmental AMR and transmission from environments to humans, from one health perspective.

This workshop is organized by:

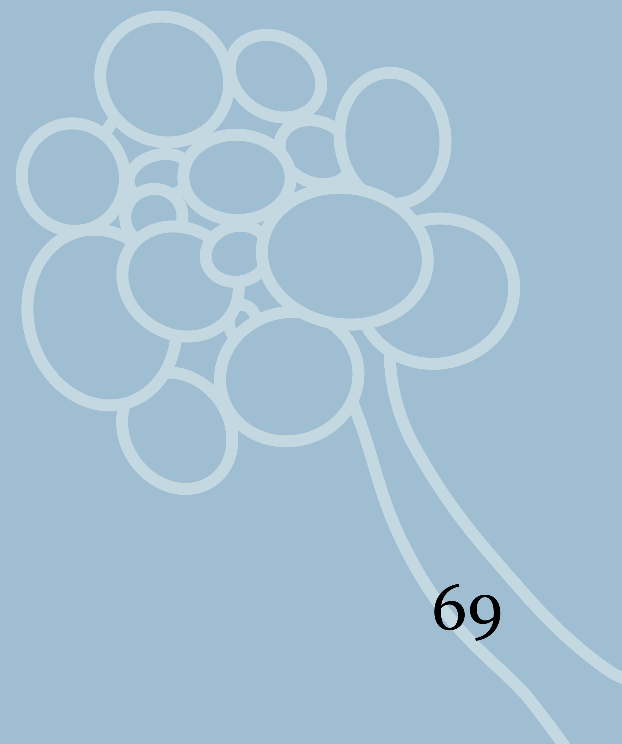
Prof. Zhugen Yang (Cranfield University, UK)
Dr. Andrew Singer (UK Centre for Ecology & Hydrology, UK)
Prof. Xin Zhao (McGill University, Canada)

Workshop 8 - Integration of genomics data, risk science, and practical considerations for conducting successful One Health AMR research

This workshop seeks to highlight real world examples of successful ‘One Health’-focused research programs that seek to integrate genomics data generated from across the One Health continuum. From a variety of scientific experts, specific and technical examples are provided related to data collection and harmonization, risk assessment and modelling, stakeholder engagement, and national/international coordination. Using the information and discussion from these presentations, a series of roundtable discussion will be guided to answer specific questions related to systemic barriers on policy uptake and communication, critical areas of focus for future EDAR research, and issues related to translation of (meta)genomics data.

This workshop is organized by:

Dr. Jordyn Broadbent (National Hydrology and Ecology Research Centre, Environment and Climate Change Canada, Canada)
Dr. Dominic Poulin-Laprade (Sherbrooke Research and Development Centre, Agriculture and Agri-Food Canada, Canada)

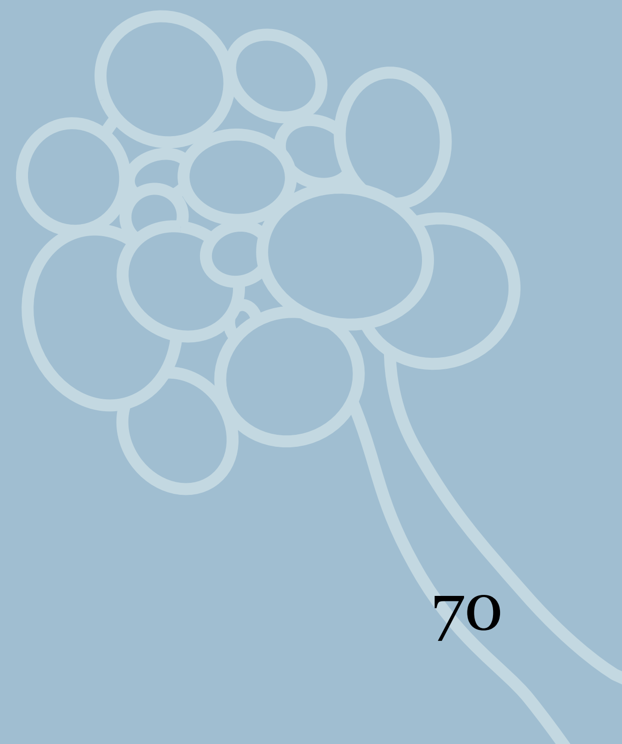


Workshop 9 - So you have AMR data, now what? Applications for actionable risk assessment and decision-making

AMR is one of the most important public health challenges of our time and monitoring the environment for AMR determinants (ARG, ARG, MGE, pathogens, etc.) as part of a “One Health” approach is aimed at informing public health decisions. Despite a wealth of environmental monitoring data (including culture-based and multi-omics data), there is a gap in translating environmental data into actionable decisions for predicting and preventing disease. This session will cover fundamental risk assessment and modeling approaches tailored to different management questions, newly developed modeling case studies with a focus on water and agricultural applications, and a participatory session to design and extrapolate integrated monitoring studies that will fill the missing linkages between causal pathways and data-driven approaches for AMR.

This workshop is organized by:

Prof. Kerry Hamilton (Arizona State University, USA)
Prof. Emily Garner (West Virginia University, USA)
Prof. Nicholas Ashbolt (Cooperative Research Centre for Solving Antimicrobial Resistance in Agribusiness, Food and Environments, Future Industries Institute, University of South Australia, Australia)
Dr. Michael Jahne (USEPA, USA)
Dr. Mary Schoen (Soller Environmental, USA)”



Workshop 10 - Resistomap - laboratory and data analysis service for environmental AMR monitoring

From Environmental Samples to Data Visualization: During this session, we will present our step-by-step standardized analysis process, from environmental sample collection to data analysis and visualization.

Resistomap is a commercial laboratory and analysis service for monitoring AMR and bacterial pathogens in various sample types, and was founded by a researcher from the EDAR community. Our services support the EDAR community by providing faster and more comprehensive results for quantifying AMR genes, without the need for an initial investment in laboratory equipment, consumables, and other resources. Furthermore, as Resistomap's intelligence platform is developed based on research results from the EDAR community, this extends the impact of research beyond the academic community.

This workshop is organized by:

Resistomap (Helsinki, Finland)
- **Dr. Windi Muziasari** (CEO)
- **Mr. William Nurmi** (CTO)
- **Mr. Ajimufti Azhari** (CoS)
- **Mrs. Alma Seppälä** (R&D Scientist)

Workshop 11 - Science for informed decision-making processes and targeted action. UN General Assembly High Level Meeting on AMR and making the economic case for AMR in the environment

This workshop will examine the costs that are likely to be imposed by the presence and flow of antimicrobials and antimicrobial resistance genes in/through the environment. It will explore what is known and what is less known, and what is not understood at all. It seeks to define future areas of research needed to fill vital gaps, and seeks to identify experts willing to continue this exploration post-conference. The workshop will also discuss the various political opportunities for bringing these issues to light and pushing forward the policy agenda.

This is an important year for AMR, especially given the interest expressed by the G7 and UN High Level meeting focusing on AMR in September. Being able to grasp some of the economic aspects related to AMs and AMR in the environment would be valuable. Getting a clearer view on what is known and what remains less certain would be helpful in creating a useful picture for these high-level discussions.

This workshop is organized by:

Dr. Chantal Morel (World Health Organisation - Quadripartite secretariat, Geneva, Switzerland)
Dr. Edward Topp (French National Institute for Agriculture, Food, and Environment [INRAE], France)

Back to the Program

Session 16
Abstract 428
Room 3-4

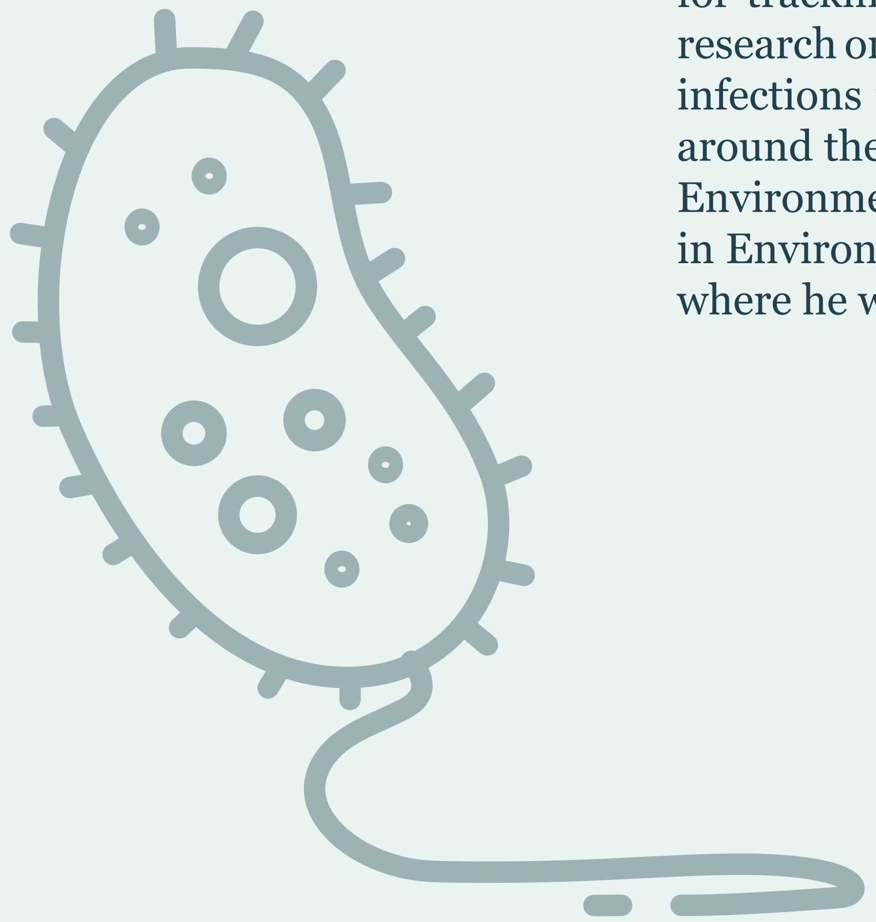
Quantifying the global impact and inequities associated with food-animal E. coli

Extraintestinal pathogenic Escherichia coli (ExPEC) strains are responsible for substantial morbidity and mortality globally. Furthermore, antimicrobial-resistant ExPEC strains are estimated to be the biggest driver of deaths directly attributable to antimicrobial resistance in low- and middle-income countries (LMICs). ExPEC strains have been isolated from humans and all the major terrestrial food animal species. However, despite the public health burden of ExPEC infections, little is known about the reservoirs of ExPEC strains. Recently we curated a global collection of E. coli genomes from humans with extraintestinal infections and food animals, screened them for 16 host-associated mobile genetic elements, then used a novel Bayesian latent class model to predict the host source of all isolates. Our model predicted that approximately 18% of human bloodstream infections (BSIs) globally were derived from food animals. The proportion of BSIs attributable to zoonotic sources were 1.7 times as common in LMICs than in high-income countries (HICs) (29% vs 17%). Likewise, extensively-antimicrobial-resistant ExPEC strains were much more common in LMICs, possibly due to poor water, sanitation and hygiene infrastructure and more intensive human-animal interactions. Extensively antimicrobial resistant ExPEC strains also appear to be much more common in LMICs; however, these findings must be viewed with caution due to the dearth of publicly-available ExPEC genomes from LMICs and the potential biases introduced by the individual studies from which they were derived. Understanding the primary sources of ExPEC infections can inform efforts aimed at reducing the heavy burden of these infections and the proliferation of antimicrobial resistance.

Author: Lance Price, George Washington University, United States

Biography:

Lance Price is a Professor at the Milken Institute School of Public Health at George Washington University and the Founding Director of the Antibiotic Resistance Action Center. Dr. Price works at the interface between laboratory research and public policy to address the growing crisis of antibiotic resistance. Dr. Price and his team are using genomics to pioneer new strategies for tracking drug-resistant pathogens and developing new strategies to stop their spread. His research on the connection between antimicrobial use in industrial agriculture and drug-resistant infections in people has been published in top scientific journals and covered by media outlets around the world. Prior to joining GW, Dr. Price directed the Center for Food Microbiology and Environmental Health at the Translational Genomics Research Institute. He received his PhD in Environmental Health Sciences from the Johns Hopkins Bloomberg School of Public Health, where he was a Center for a Livable Future Fellow.



Session 16
Abstract 19
Room 3-4

Antibiotic ecotoxicity and resistance risks in chicken and pig farming environments in Kenya

Background: In Low- and Middle-Income Countries (LMICs) like Kenya, the burgeoning livestock industry plays a pivotal role in economic development and food security. However, the widespread use of antibiotics in livestock farming has precipitated a hidden crisis: emergence of antibiotic-resistant bacteria and antibiotic ecotoxicity but data from these agroecosystems are limited.

Method: We studied semi-intensive chicken (n=52) and pig (n=47) farms in a resource-limited setting to understand antibiotic usage, manure management practices and measure risk levels of antibiotics in the manure-soil pathway. We validated an LC-MS/MS method for analyzing eight commonly used veterinary antibiotics and quantified AMR risks.

Findings: Chicken compost (25.8%, n=97/376) had the highest antibiotic prevalence, followed by pig manure-fertilized soils (23.1%, n=83/360). In general, the average antibiotic concentration in environmental samples was low (63.4 μg/kg), except trimethoprim (221.4 μg/kg) and pig manure-fertilized soil (129.3 μg/kg), which exceeded the environmentally relevant threshold (i.e.,100 μg/kg). The dominant antibiotics by concentrations were tetracycline, oxytetracycline (chicken system), and trimethoprim (pig system). The additive AMR risks in the farms were low (risk quotient < 0.1), and differences between sample types and farms were not statistically different ($p>0.05$). However, the likelihood of ecotoxicity risks in pig systems was significantly higher ($p<0.05$) than in chicken systems. Ecotoxicity and AMR selection risks increased with the chicken flock size, and ecotoxicity increased with the number of antibiotics used in pig systems. Even though AMR risks were low ($RQ<1$), they pose noticeable risks that require additional assessment, including evaluating mixtures of antibiotic residues.

Implication: Our study provides data on antibiotic residue levels in semi-intensive livestock farms and shows how a deterministic risk assessment is applicable for non-intrusive AMR surveillance. Further large-scale studies on ecotoxicity and AMR risks are necessary for evidence-based pollution control and environmental AMR mitigation.

Author: Fredrick Gudda, International Livestock Research Institute, Kenya

Co-Authors: Moodley, Arshnee; Muloi, Dishon; Gao, Yanzheng

Back to the Program

Session 16

Abstract 273

Room 3-4

Strategies to Combat Antibiotic Resistance Genes Using Magnetic Biochar/Quaternary Phosphonium Salt in Agricultural Systems

The farmland ecosystem is an important reservoir for antibiotic resistance genes (ARGs) to spread to human beings. The irrigation with sewage and farmland application of manure or biosolids in agricultural practices introduce substantial selective agents such as antibiotics and toxic metals, aggravating the transfer of ARGs from the soil environment to humans via the food chain. Strategies to combat ARG dissemination are pressingly needed for human and ecological safety. Here, a newly synthesized biochar-based polymer - magnetic biochar/quaternary phosphonium salt (MBQ) - was developed using precipitation of iron oxide on biochar followed by ion exchange with quaternary phosphonium salt, and used to combat the proliferation and spread of ARGs. MBQ could effectively achieve bacterial deactivation and extracellular ARGs adsorption under culture conditions by the synergistic effect of oxidative damage arising from the formation of •OH, the intercalation of MBQ colloid, and the interaction with released cationic QPS. MBQ was applied in livestock wastewater and displayed a high performance in bacterial deactivation (99%) and ARG decrease (up to 4 orders of magnitude). Furthermore, foliar spraying with MBQ reduces the abundance of “high-risk ARGs” and pathogens in leafy crops, lowering the risk of ARGs entering the food chain. With the advantages of effective, recyclable, and long-acting antibacterial capabilities, MBQ has great potential in many fields such as sanitation, medical care, and environmental remediation, and is proposed as a new functional material to reduce the health risks caused by ARGs in agriculture environments.

Author: Fang Wang, Institute of soil science, China

Session 16

Abstract 265

Room 3-4

Constructed wetlands as a nature-based solution for removing antibiotics and antibiotic resistance genes from wastewater

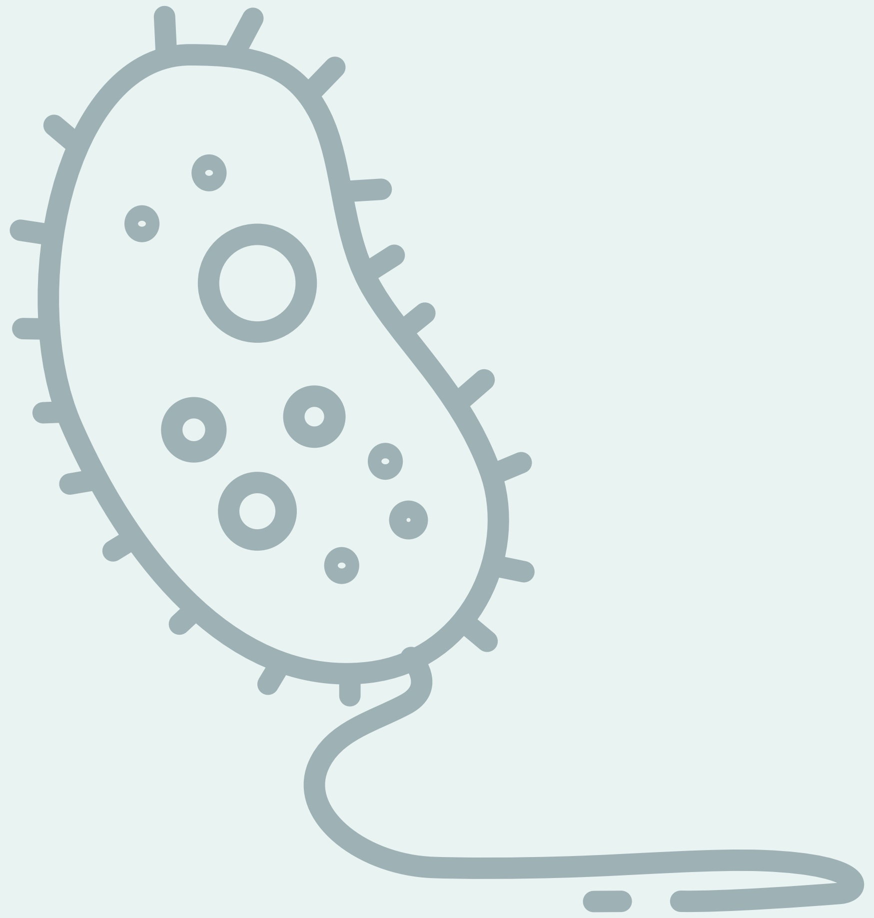
Background and Aim: Constructed wetlands (CWs) are efficient and frequently implemented nature-based solutions for wastewater treatment. However, how such systems deal with contaminants of emerging concern such as antibiotics and antibiotic resistance genes (ARGs) is still unclear. Works on the performance of full-scale systems over long monitoring cycles are still scarce. To address these gaps, a full-scale CW has been studied for one year to gain knowledge on real-life efficiency and seasonal performance.

Method: the selected full operation CW provides decentralized wastewater treatment for a small village (100 PE). Water and porous media were sampled to analyse organic carbon and nitrogen, microbial community (NGS), organic micropollutants (including antibiotics) and ARGs (qPCR).

Findings: the CW was efficient in nutrient removal and reducing organic micropollutants, including antibiotics and ARGs. Few antibiotics were sporadically quantified, however ARGs were always detected. The removal efficiency for organic micropollutants was influenced by seasonality (lower in winter than autumn, spring and summer), presumably due to the different P. australis vegetative states during the year. The sul1, sul2 and IntI1 genes were detected in all microbiological samplings. Interestingly, the vertical flow bed (1st of the two-stage CW) resulted very effective in decreasing both emerging contaminants and ARGs. The prokaryotic community in the vertical and aerated (2nd-stage) beds were significantly more biodiverse than that of flowing water, showing how plants promoted a stable rhizospheric microbiome. Bacteria associated with N-cycle, P removal and antibiotic degradation (e.g. Pseudomonas and Flavobacterium) were found. Finally, several pathogens and potentially antibiotic resistant (e.g. Acinetobacter, Aeromonas, Arcobacter, Comomonas, Pseudarcobacter, Saccharimonadales) decreased significantly from the inlet to the outlet water. This full-scale CW demonstrated all year-round capacity to remove antibiotics and ARGs from wastewater.

Applications: CWs can be considered a promising technology for halting antibiotic and ARG spread and their application at larger scale is desirable.

Author: Anna Barra Caracciolo, Water Research Institute - National Research Council (IRSA-CNR), Italy



Back to the Program

Session 16

Abstract 386

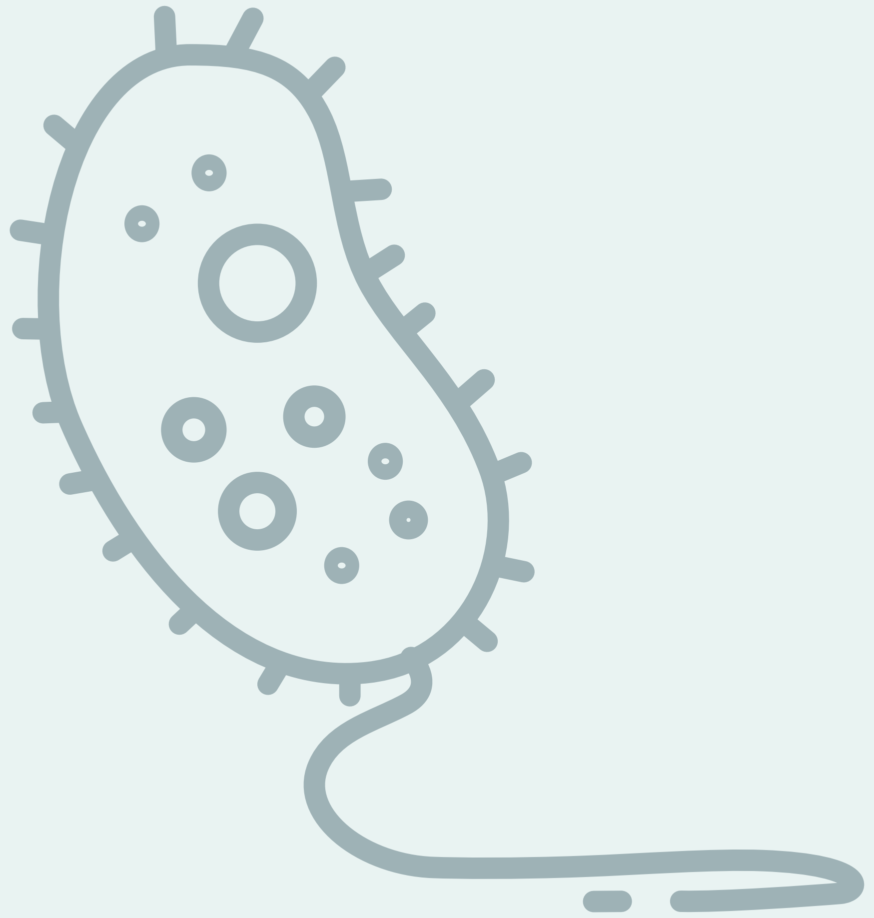
Room 3-4

Environmental antimicrobial resistance threats to food safety and security

OBJECTIVE: Antimicrobial use in food animals contributed to the emergence of Antimicrobial Resistance (AMR) in the environment and contamination of produce. Honey and pollen are a reliable bioindicators of environmental AMR. This study aims to infer the mechanisms of AMR spread from the environment to produce and its pollinator, and the risk of pollinator disease due to exposure to AMR following the use of composted or raw poultry litter as a fertilizer.
METHODS: In this controlled field experiment, two high tunnel greenhouses were divided into 3 subsets. Yellow squash was planted and fertilized with either fresh or composted poultry litter. Apis mellifera honey bee colonies were introduced when squash plants flowered. Soil, pollen, honey bee, and produce samples were collected every week for 5 weeks. The relative concentration of antimicrobial genes (ARGs), and mobile genetic elements (MGEs) were compared using qPCR. Antimicrobial residue was extracted and measured using Mass Spectrometry.
RESULTS: Preliminary analysis revealed higher levels of ARGs and MGEs in baseline samples of raw poultry litter compared with composted. A progressive increase in ARGs was observed over time in soil fertilized with raw poultry litter compared with composted. There is evidence of spread of ARGs from fertilized soil to pollen, produce, and honey bees irrespective of the fertilizer used. However, differences between samples exposed to composted or poultry litter were inconsistent between sample types and will be further explored.
IMPLICATIONS: When untreated production animal waste is used as fertilizer, residues and AMR accumulate in the environment, contaminating fresh produce, and potentially contributing to dysbiosis and higher predisposition to diseases in honey bees. This project provides evidence to understand the ecological interconnections between food production, farm environment, and pollinators relevant to AMR spread and its impact on food safety and the ecosystem's health.

Author: Kiranmayee Bhimavarapu, Auburn University, United States

Co-Authors: Huber, Laura; Williams, Geoffrey; Rodrigues, Camila; da Silva, Andre Luiz; Boersma, Melissa



Thursday, May 30 - 11:00-12:30

Back to the Program

Session 17

Abstract 427

Room 4

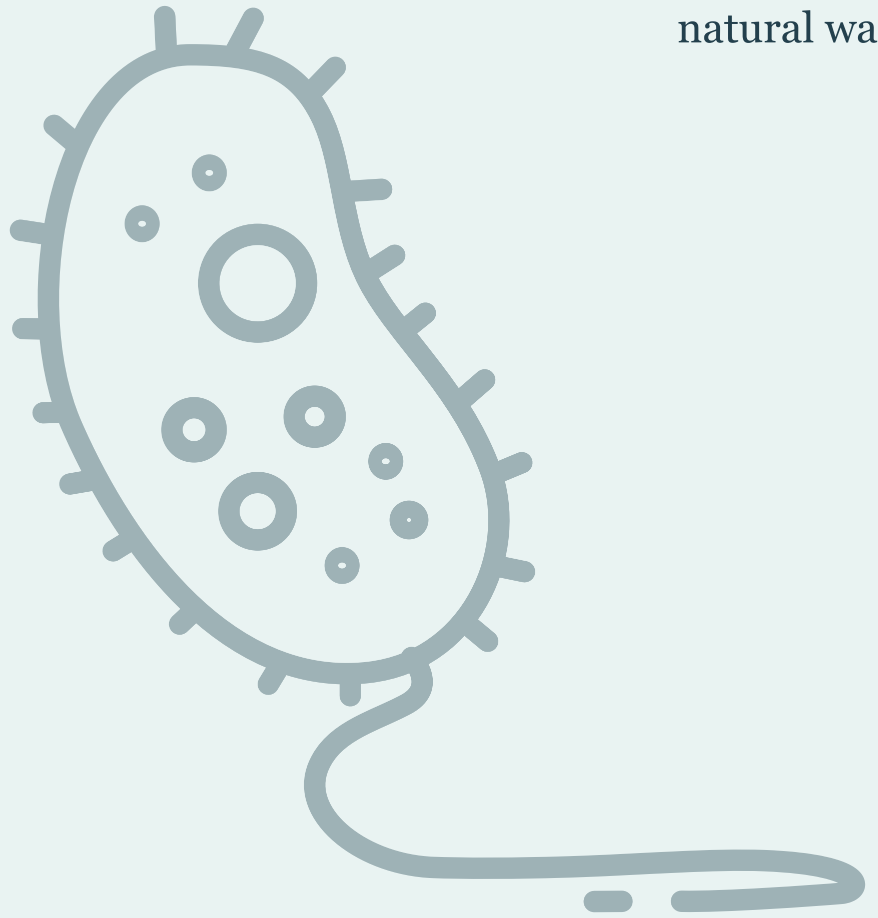
AMR and pathogen evolution in a One Health context

Metagenomics provides unparalleled insights into the genetic composition of diverse microbial populations. The technology has revolutionised understanding of antimicrobial resistance (AMR) and is the methodology of choice for measuring the impact of AMR mitigation strategies, hunting latent AMR genes, and defining AMR in diverse hosts, environments and surfaces. However, one the greatest threats afforded by AMR is the relentless rise of multiple drug resistance (MDR) in the ESKAPE-E pathogens. Rather than metagenomics, whole genome sequencing is unparalleled as a method to study AMR and evolution of ESKAPE-E pathogens. Escherichia coli is an ESKAPE-E organism continually released from the gut of vertebrates species into the environment, pooling in agricultural and municipal wastewater systems and natural water bodies. Distinct lineages cause intestinal and extraintestinal infections (ExPEC) in humans, major food-producing and companion animals, and is a major driver of antimicrobial use globally. Hybrid E. coli pathovars are reported frequently, but with poor predictability, and occasionally with devasting clinical consequences. ExPEC are the most frequently isolated Gram-negative bacterium, responsible for urinary tract and bloodstream infections and was the number one cause of deaths associated with AMR in 2019, a trend expected to continue in coming decades. The ecology of E. coli, particularly in environmental settings, remains poorly described. The role of mobile genetic elements in AMR cannot be determined without accurately assembled, complete genome sequences. These knowledge gaps must be addressed if genomic surveillance systems are to reliably predict emerging infectious and MDR threats before becoming established in clinical settings.

Author: Steven Djordjevic, University of Technology Sydney, Australia

Biography:

Steven P. Djordjevic is a Distinguished Professor of Infectious Disease in the Australian Institute for Microbiology &amp; Infection at the University of Technology Sydney. His research interests are in antimicrobial resistance and pathogen evolution through a One Health lens. His laboratory conducts genomic epidemiological analyses of a broad range of Gram negative bacterial pathogens, and the mobile elements they carry, from hospital and veterinary clinical environments, food production animals, synanthropically-impact wildlife, wastewater and natural water systems.



Session 17

Abstract 133

Room 4

Dangerous hitchhikers! - How cigarette filters promote the spread of antimicrobial resistant bacteria in the environment.

Background and Aim: While immediate health risks of smoking are well-established, indirect health impacts of cigarette-derived pollutants through promoting the proliferation of AMR remain understudied. With the huge consumption of 9 trillion cigarettes, around 1.2 million tons of filters are produced and discarded annually, together with the entrapped toxicants, making them an important environmental pollutant. Furthermore, their large surface area makes them a perfect breeding ground for microbial colonization. We here propose that the toxic compounds entrapped in cigarette filters and leaching from filters, ashes and smoke (co-) select for antimicrobial resistance and promote plasmid transfer. This results in high-risk filter-colonizing communities which can then hitchhike large distances to novel environments.

Procedure/Method: To test this, used cigarette filters enriched in toxic residues and unused control filters were submerged in a wastewater receiving stream and their colonizing microbial communities were analyzed for AMR, mobile genetic elements (MGEs) and pathogenicity. We furthermore performed plasmid transfer experiments between Pseudomonas strains in river water medium, in the presence of different concentrations of cigarette filter and cigarette ash leachate.

Findings/Results: Used cigarette filters enriched in toxic residues were selectively colonized by microbial communities significantly and consistently enriched in pathogenic potential and >30 tested ARGs and MGEs compared to unused filters. Furthermore, toxic leachates from cigarette filters and ash resulted in >2-fold elevated transfer rates of a multi-drug-resistance encoding plasmid. Similarly, cigarette smoke condensate at concentrations equivalent to the consumption of 8 cigarettes daily significantly elevated plasmid transfer rates in lung sputum medium. These effects were connected to an activation of bacterial stress responses when exposed to cigarette-derived toxicants.

Implications/Applications: Discarded cigarette filters, that often travel long distances in our waterways can facilitate the hitchhiking of high-risk bacteria to novel environments. Furthermore, cigarette-derived compounds can promote the spread of AMR within the human lung and natural environments.

Author: Uli Klümper, TU Dresden, Germany

Co-Authors: Fang, Peiju; Konyali, Diala; Li, Bing; Dalpke, Alexander; Huang, Jin; Berendonk, Thomas

Thursday, May 30 - 11:00-12:30

Back to the Program

Session 17

Abstract 200

Room 4

The impact of various microplastics on bacterial community and antimicrobial resistance genes in Norwegian and South African wastewater

Background and Aim: Wastewater treatment plants (WWTPs) may serve as a hotspot for pathogens and promote antimicrobial resistance (AMR). Plastic debris in wastewater could further contribute to AMR dissemination.

The aim of this study is to investigate the impact of various microplastic types on bacterial communities and AMR gene abundance in Norwegian and South African wastewater.

Method: Microcosm experiments were designed as follows. Five manufactured microplastic pellet types were used for testing and two rock aggregate types were used as controls. In addition, each material type was subjected to artificial aging treatments using either ultra-violet light or hydrogen peroxide.

Each material was incubated in flasks containing inlet/outlet wastewater obtained from Norwegian/South African WWTPs statically at room temperature. Nucleic acids were extracted after a one-week incubation period.

The detection of the blaFOX and blaMOX genes was performed using quantitative PCR. Extracted DNA was sequenced using a MinION device.

Results: Non-metric multi-dimensional scaling plot on full-length 16S sequencing data at the species level showed samples were clustered into distinct material groups. These results were in line with the ANOSIM test showing significant p-values in both Norwegian and South African WWTP settings. Indicator species analysis showed a strong association between many Acinetobacter species with the plastic group. Aging treatment did not show apparent effects on microbial composition.

The abundance of blaFOX and blaMOX genes in the Norwegian wastewater outlet were generally lower compared to those in the inlet, though results were contrary in South African wastewater samples. The relative abundance of AMR genes seemed to be increased on several plastic types (PET, PE, PLA) but decreased on PVC-A.

Implications: WWTPs in this study did not effectively reduce the abundance of AMR genes. In addition, plastic categories were shown to play a pivotal role in developing distinct bacterial communities and AMR profiles.

Author: Tam Thanh Tran, NORCE, Norway

Co-Authors: Stenger, Kabelo Stephans; Bezuidenhout, Carlos Cornelius; Strømmen, Marte; Wikmark, Odd-Gunnar

Session 17

Abstract 309

Room 4

Cyanobacterial Blooms are Antibiotic-Resistant Gene Reservoirs in Drinking Water Sources: Potential Public Health Impacts Beyond Cyanotoxins

Background and Aim: Freshwater harmful cyanobacterial blooms (HCBs) are expanding across the world, and their frequency and severity become more intensified due to prevalent eutrophication and changing climate. Traditionally, the concerns about HCBs have been mainly focused on cyanotoxins in water. Recent studies reveal the potential interplay of antibiotic resistance (AR) and cyanobacteria, but hardly any study has been done to investigate the role of cyanobacteria as hosts of AR genes and environmental AR reservoir in drinking water sources. The main goal of this study was to characterize the resistome and microbial community and to determine whether cyanobacteria host ARGs and whether these ARGs are mobile and clinically relevant.

Procedure/Methods: Water samples were collected three times in 2022 from water intake locations at the Toledo drinking water treatment plant (Lake Erie) and Celina drinking water treatment plant (Grand Lake St. Marys [GLSM]) in Ohio, United States. Molecular methods such as droplet digital PCR and toxin detection, and shotgun metagenomic sequencing were used to analyze cyanobacteria, ARGs, microbial communities, and resistome.

Findings/Results: The results showed high levels of Microcystis followed by Aphanizomenon in Lake Erie, and Planktothrix in GLSM, and microcystin in GLSM. A high environmental resistome was observed in both water sources. The major annotated ARG classes for both locations are multidrug, tetracycline, aminoglycoside, and bacitracin. Cyanobacteria were suggested to host ARGs (Lake Erie: Microcystis, Pseudanabaena, and Vulcanococcus; GLSM: Planktothrix) and rpoB2, arlR, and vatB are the major genes, and OXA and emrE were also annotated on cyanobacteria.

Implications/Applications: The results indicate that cyanobacteria host ARGs and imply that HCB-impacted water not only has cyanotoxin concerns but also increases AR burden. Advanced water treatment procedures, such as biological activated carbon, for cyanotoxin treatment can reduce the toxin, but it may actually encourage ARG transmission between bacteria.

Author: Jiyoung Lee, Ohio State University, United States

Thursday, May 30 - 11:00-12:30

Back to the Program

Session 18
Abstract 280
Room 3

Concentrations in WWTP influents mirror fecal discharge and community carriage of resistant bacteria

Background and Aim: Wastewater based epidemiology (WBE) is a tool to monitor infectious disease outbreaks, but could ideally also be used to estimate carriage of antibiotic resistant bacteria in the human general community. However, quantitative estimates of community prevalence require knowledge on the correlation of concentrations of resistant bacteria in wastewater with the community prevalence. The aim of this study was to evaluate to what extent bacterial concentrations in WWTP influents could be predicted with mathematical modelling.

Procedure/Method: Concentrations of resistant bacteria in WWTP influent were derived from mathematical modelling, based on the number of people connected to and the daily water flow of WWTPs, fecal bacterial concentrations of Escherichia coli (E. coli), extended-spectrum beta-lactamases-producing E. coli (ESBL-EC), and carbapenemase-producing Enterobacterales (CPE), and the daily amount of feces produced per person, at a given – known – prevalence in the population. These were compared to measured concentrations obtained at >100 WWTPs.

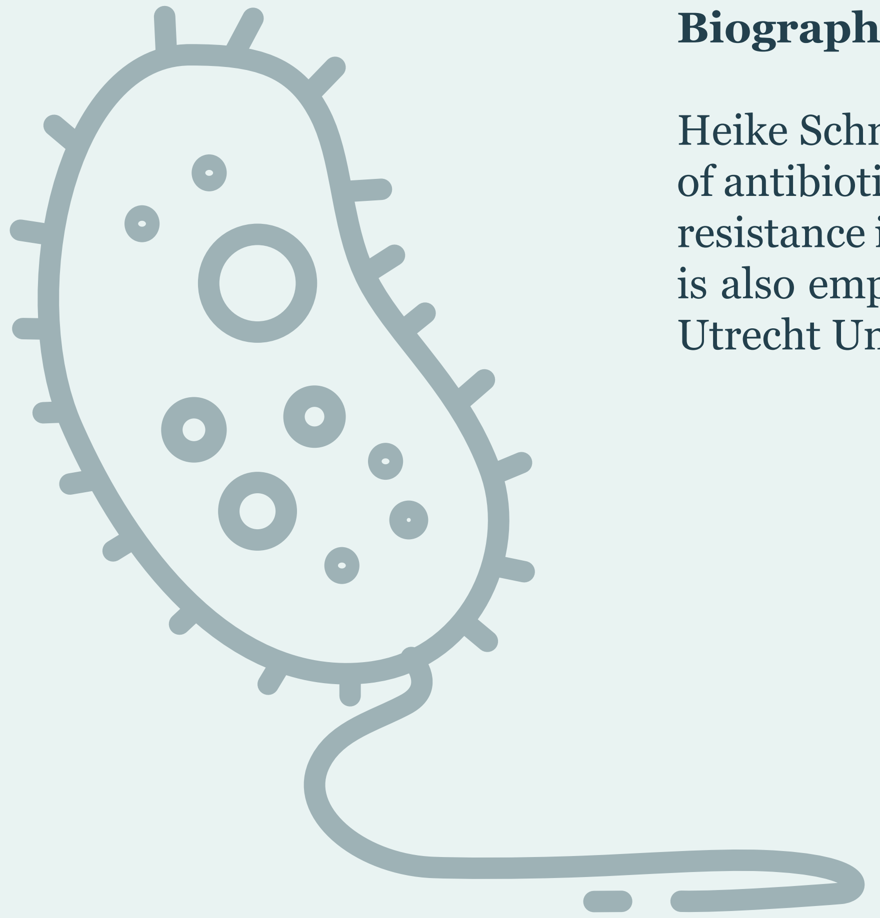
Findings/Results: Wastewater concentrations of E. coli, ESBL-EC, and CPE were predicted by model simulations with reasonable precision (measured concentrations were underestimated by 0.1-0.4 log10 CFU/L). For single WWTP sampled repeatedly, the range of concentrations determined by the model was similar to that of repeated samplings. The variation in modelled concentrations is mainly caused by stochastic events (due to highly variable fecal concentrations of resistant bacteria) and enhanced by low community prevalence (due to the effect of chance on the presence or absence of one single carrier in a WWTP catchment). Therefore, sampling at larger WWTP reduces measurement variability.

Implications/Applications: The model can now be applied to determine the sensitivity of wastewater measurements to detect trends in wastewater concentrations, and to develop effective sampling schemes. Last, it bears promise to derive community carriage once the divergence between model outcomes and experimental findings has been further resolved.

Author: Heike Schmitt, National Institute for Public Health and the Environment (RIVM), Netherlands

Biography:

Heike Schmitt is senior advisor to the theme “source separated sanitation”, on the transmission of antibiotic resistance in the environment. In 2022 she was appointed as professor of Antibiotic resistance in the water cycle, department of Biotechnology at Delft University of Technology. She is also employed by the National Institute for Public Health and the Environment (RIVM) and Utrecht University.



Session 18
Abstract 156
Room 3

Resistance in sewage-associated microbiota mirror patterns of clinical isolates and reflect a variable lagging association with antibiotic usage

Background and Aim: Conventional monitoring of antibiotic resistance is heavily reliant on centralized clinical infrastructure for the primary purpose of guiding patient care. However, clinical surveillance is inherently limited in its ability to yield information regarding the full range of pathogens relevant to AMR. Metagenomics informed wastewater-based surveillance (WBS) has the potential to complement clinical surveillance efforts as a means of characterizing resistance patterns at the community-level, but there is a need to establish the relationship between antibiotic resistance gene (ARG) abundance in sewage and clinical resistance.

Procedure/Method: Here we integrate community antibiotic usage data and a year-long prototype WBS system at the corresponding wastewater treatment plant (WWTP). Composite samples of influent and final effluent were collected twice weekly between August 2020 and September 2021 and subjected to deep metagenomic sequencing (~36 Gbp/212 million reads per sample). Collaboration with local clinicians provided outpatient antibiotic prescription data that covered the majority of antibiotic prescriptions from 2017-2022 within the sewershed. The resulting dataset provided an unprecedented opportunity to model wastewater antibiotic resistance in relation to both physical-chemical and usage parameters in a single community.

Findings/Results: Nested statistical models revealed that, when controlling for the sewage microbial characteristics, antibiotic usage had a limited impact on drug class relative abundance in influent (between 2-8% increased variance explained). However, analysis of individual ARGs revealed a striking association with antibiotic usage. It was found that a subset of ARGs, predominantly associated with Enterobacteriaceae, displayed a direct correlation with antibiotic usage, while ARGs predominantly associated with Pseudomonadaceae displayed a lagged relationship with antibiotic usage (between 1-3 months). Comparison of the correlation results with antibiogram data suggested microbial ecological drivers of the observed trends.

Implications/Applications

The study described herein advances understanding of the impact of antibiotic use on wastewater.

Author: Connor Brown, Virginia Tech, United States

Co-Authors: Rumi, Monjura Afrin; McDaniel, Lauren; Maile-Moskowitz, Ayella; Sein, Justin; Choi, Minyoung; Hindi, Fadi; Emon, Muhit; Ahmad Moumi, Nazifa; Blair, Matthew; Rao, Jayashmina; Baffoe-Bonnie, Anthony; Vikesland, Peter; Pruden, Amy; Zhang, Liqing

Back to the Program

Session 18

Abstract 179

Room 3

Culture enriched wastewater exhibits an increased burden of antibiotic resistant genes in hospitals relative to the community

Background and Aims: Surveillance of antimicrobial resistance (AMR) is crucial to both understanding its magnitude and the factors contributing to its prevalence. Wastewater-based surveillance enables objective, comprehensive and inclusive assessments of AMR across entire populations served by shared sewersheds. This work aimed to determine if the resistome of hospitals as measured in its wastewater differed from that of the general population, and whether selective culture enrichment could enhance the detection of specific AMR genes.

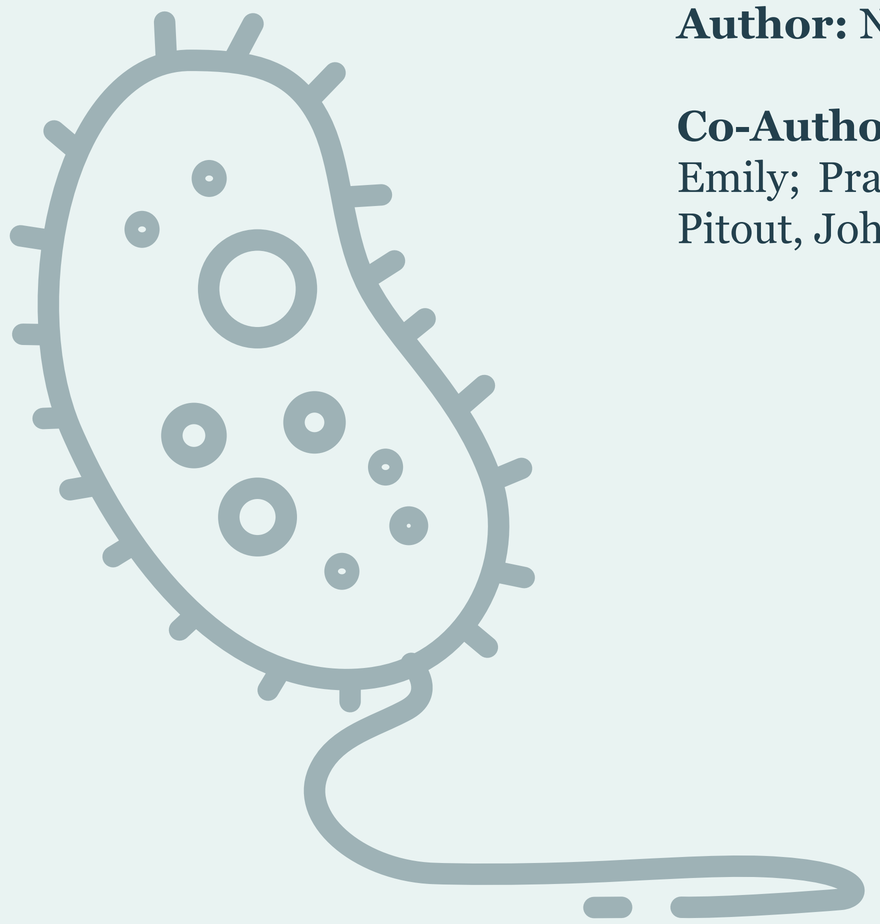
Procedure/Method: We compared AMR genes in 24-hour composite wastewater samples collected from two tertiary-care hospitals and two urban neighborhoods. We used complementary targeted qPCR and metagenomics analysis on wastewater, both before and after selective culture enrichment targeting clinically important bacteria (using MacConkey agar impregnated with sub-inhibitory levels of antibiotics targeting Gram-negatives). Total genomic DNA extraction was performed on raw wastewater pellets or culture-enriched pellets, followed by Illumina sequencing and DeepARG-SL (v2.0) analysis.

Findings/Results: A total of 1225 AMR genes were identified in 20 samples, with β-lactam resistant genes showing the greatest diversity, followed by multidrug, fluoroquinolone, macrolide-lincosamide-streptogramin, and aminoglycoside. The composition profiles of AMR genes in wastewater differed before and after culture enrichment ($p<0.005$). Hospital wastewater exhibited higher diversity and abundance of AMR genes compared to neighborhood samples measured as raw ($p<0.05$), and after enrichment ($p<0.05$). Similar results were found when different AMR genes of interest were analyzed using qPCR. Clinically relevant AMR genes like VIM and NDM metallo-β-lactamases were not detected in raw wastewater pellets, but detected after enrichment, even with low sequencing depth.

Implications/Applications: The results highlight the effectiveness of employing multiple approaches —metagenomics, semi-selective culture enrichment, and qPCR— in comparing resistomes between locations and improve the identification of ultra-rare, clinically relevant AMR genes. Wastewater-based surveillance has substantial potential in enhancing the impact of hospital-based infection prevention and control, and antimicrobial stewardship programs.

Author: Nicole Acosta, University of Calgary, Canada

Co-Authors: Lee, Jangwoo; Bautista, Maria A.; Bhatnagar, Srijak; Waddell, Barbara J.; Au, Emily; Pradhan, Puja; Clark, Rhonda G.; Meddings, Jon; Ruecker, Norma J.; Achari, Gopal; Pitout, Johann; Conly, John; Frankowski, Kevin; Hubert, Casey; Parkins, Michael D.



Session 18

Abstract 125

Room 3

From clinics to sewers: leveraging environmental surveillance to inform epidemiology of clinically-relevant antibiotic resistant bacteria

Background/Aim: Carriage of antimicrobial resistance (AMR) within communities is a risk factor for antimicrobial resistant infections. AMR trends are primarily monitored in clinical settings, but this proves challenging due to large sample sizes, high associated costs, and privacy concerns. Wastewater monitoring offers insights into AMR across populations, with antibiotic resistance trends in wastewater potentially informing clinical patterns.

Procedure/Method: We quantified six clinically-relevant antibiotic resistant bacteria (ARB) in Swiss wastewater influent from six wastewater treatment plants (WWTPs) weekly. Using culture-based methods, we processed 582 wastewater samples between November 2021 and November 2023 to enumerate: extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae (ESBL-Ec and ESBL-Kp), carbapenemase-producing E. coli and K. pneumonia (CP-Ec and CP-Kp), methicillin resistant Staphylococcus aureus (MRSA), and vancomycin resistant Enterococcus faecium/fecalis (VRE). ESBL-Ec detection began in November 2021; additional targets were included in December 2022. We further performed whole genome sequencing (WGS) on 826 ESBL-Ec isolates from wastewater, hospitalized patients, wildlife and livestock feces collected across Switzerland over a year.

Findings/Results: ARB were detected in ≥82% of wastewater samples. Median percentages of ARB over total bacteria (resistant plus susceptible) were 0.05% (CP-Ec), 0.06% (CP-Kp), 0.09% (VRE), 0.3% (ESBL-Kp), 1.6% (ESBL-Ec) and 16.3% (MRSA). Significant differences in percentages were observed among WWTPs and over time. We leveraged two years of ESBL-Ec percentages data in wastewater to build a mechanistic model linking wastewater percentages to carriage prevalence within the population. The linkage gains clarity when considering the proportions of ESBL-Ec in the guts of carriers. WGS determined the genetic relatedness and migration of ESBL-Ec between clinical settings, the environment and wastewater, revealing transmission dynamics in Switzerland in a “One Health” approach.

Implications/Applications: Our study demonstrates wastewater-based surveillance of culturable ARB and provides reliable insights into spatial and temporal trends, establishing foundations for national monitoring and targeted interventions, independent of clinical surveillance.

Author: Sheena Conforti, EAWAG, ETH Zürich, Switzerland

Co-Authors: Holschneider, Aurélie; Habluetzel, Camille; Sylvestre, Émile; Du Plessis, Louis; Stadler, Tanja; Julian, Timothy R.

Thursday, May 30 - 13:45-15:30

Back to the Program

Session 19

Abstract 163

Room 4

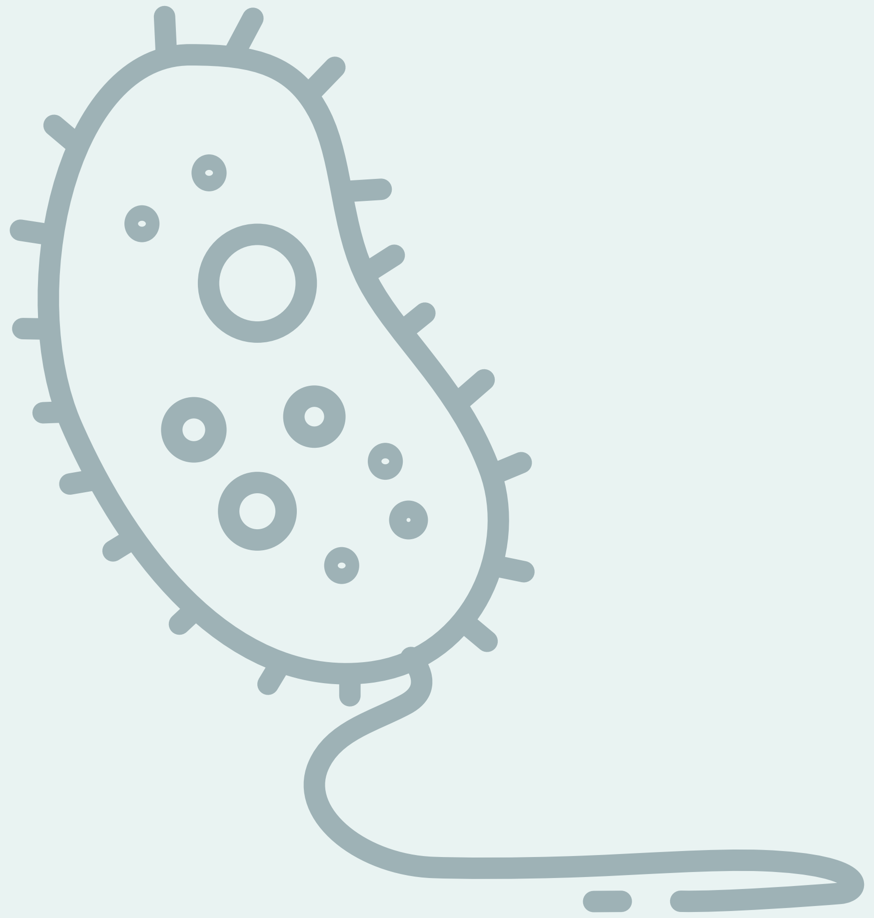
Wildlife in transmission of antimicrobial resistance

Resistant microbes selected by antibiotic use in the human and agriculture sectors subsequently spread to the environment and reach wildlife living in human-influenced habitats. The occurrence of antimicrobial resistance (AMR) in wildlife is a complex problem influenced by various biological, ecological and geographical factors which are not yet fully understood. Urbanization greatly influences breeding and feeding habits of certain wildlife species, endorsing connections between wildlife and humans and supporting the cross-transmission of AMR. Resistant bacteria were observed in various species of wild animals around the globe, which were proved to carry multi-drug resistant bacteria, high-risk human clones and genes conferring resistances to last-line antibiotics such as carbapenems or colistin. Although many studies documented the dissemination of AMR into wildlife, they also raised important gaps in our understanding of the role of wild animal species in the complex transmission pathways of resistant microbes. Most of these studies focused on wildlife as sentinels affected by our activities that show the level of environmental contamination by AMR, but fewer studies aimed to examine wildlife as vectors or even amplifiers of AMR. Although wildlife is probably not a main transmission route of AMR, it represents an important pathway of resistant microbes in the environment. Various aspects of AMR in wild animals as well as current knowledge gaps will be discussed in the presentation.

Author: Monika Dolejska, University of Veterinary Sciences Brno, Czechia

Biography:

Monika Dolejská is an Associate Professor in Microbiology at University of Veterinary Sciences Brno and Charles University, Czech Republic. She specializes in molecular epidemiology of antimicrobial resistance in Escherichia coli from animals, the environment and wildlife. Her research focuses mainly on dissemination of bacteria with plasmid-mediated resistance genes to critically important antimicrobials, such as cephalosporins, carbapenems and fluoroquinolones and comparative sequence analysis of antibiotic resistance plasmids and high-risk E. coli clones from diverse sources.



Session 19

Abstract 90

Room 4

Evils and allies: gulls as spreaders and sentinels of Antibiotic Resistant Bacteria in human-transformed environments

Background and Aim: Human-transformed environments are involved in the emergence of Antibiotic Resistant Bacteria (ARB), especially human waste production (dumps), agricultural and livestock production. Wildlife (e.g. gulls) can act as vectors for dispersal through different environments, but also can act as sentinels to detect the early circulation and anticipate the risks derived from ARB to humans and livestock. Procedure/Method: We provide a model study based on GPS tracking information, clinical testing and network analyses from the yellow-legged gull that shows the spatial patterns of ARB dispersal within an agricultural context. We tested and tagged 21 breeding individuals during 2022 and 2023. Findings/Results: Six of them tested positive for Escherichia coli (one of them was co-infected with Listeria monocytogenes and Salmonella spp.). Potential pathogen dispersal distances were 1.16 km from the breeding colony, with maximum distances of 23.17 km. We also created a connectivity network based all 21-tagged individuals to determine the main sources of ARB in the area (associated to three habitats: dumps, livestock facilities and irrigation ponds). The network included 54 nodes and 975 links and showed a high connectivity between the lake and the nearby dumps. Implications/Applications: The combination of GPS movement data, network analyses, and pathogen determination to create spatial risk maps and networks for ARB sources can become an important tool for managing human-transformed ecosystems and improving animal health and public health with a One Health approach.

Author: Víctor Martín Vélez, Marine Sciences Institue-CSIC, Spain

Co-Authors: Montalvo, Tomas; Gimenez, Joan; Ramirez, Francisco; Sarda-Palomera, Francesc; Santisteban, Carlos; Sabate, Sara; Planell, Raquel; Morral-Puigmal, Clara; Giralt, David; Bota, Gerard; Navarro, Joan

Back to the Program

Session 19
Abstract 335
Room 4

Zooplankton affects extracellular DNA degradation and uptake by natural transformation in freshwater microcosms

Background and Aim: Extracellular DNA (eDNA) is commonly regarded as pivotal in spreading antibiotic resistance genes (ARGs) in freshwater environments. Nevertheless, the fate and uptake of eDNA in this habitat have been poorly investigated in the food-web context, i.e. considering the bacterial interactions with higher organisms such as zooplankton. Here, we aimed to evaluate the impact of zooplankton presence on eDNA persistence and uptake by bacteria through natural transformation.

Method: Experiments were carried out in microcosms of artificial lake water. Quality and quantity of eDNA were evaluated in presence of the zooplankton Daphnia obtusa or the water in which Daphnia was allowed to release compounds among which proteins. Released proteins was identified through liquid chromatography-high resolution mass spectrometry. Uptake of eDNA by natural transformation was assessed in experiments with Acinetobacter baylyi BD413 selected as a model of naturally competent bacteria.

Results: While in presence of alive individuals of D. obtusa, plasmidic and linear DNA was degraded, the addition of water in which Daphnia was previously incubated showed a gradual modification of plasmid conformation following an animal concentration-dependent trend. The identification of functions related to DNA binding and degradation for Daphnia-released proteins suggested the involvement of Daphnia and its associated microorganisms on eDNA degradation and topology modification. Natural transformation experiments showed a low frequency in presence of Daphnia individuals, due to plasmid degradation, and a high frequency in presence of Daphnia-released molecules, possibly due to the modification of plasmid conformation. In conclusion, data showed that zooplankton and its microbiota influence eDNA degradation and topology thus affecting eDNA uptake by bacteria.

Implications

This study suggests that the zooplankton and its microbiota could have a role in the diffusion of antimicrobial resistance in freshwater.

Author: Elena Crotti, University of Milan, Italy

Co-Authors: Riva, Francesco; Ferrari, Emanuele; Lazzaro, Federico; Borin, Sara; Di Cesare, Andrea; Mapelli, Francesca; Eckert, Ester

Session 19
Abstract 259
Room 4

Impact of wastewater effluents on the carriage of antibiotic resistant bacteria and genes in cattle grazing downstream wastewater treatment plants.

Background and aim: In certain areas, cattle used for food production are in contact with contaminated surface waters located downstream of wastewater treatment plants (WWTPs). Thus, there is a potential risk for transfer of genes encoding antibiotic resistance and antibiotic resistant bacteria from contaminated surface waters to cattle, but also vice-versa. In the current study, as part of the PAIRWISE project, we examined if cattle in direct contact with contaminated water have a higher risk of AMR carriage.

Method: We sampled herds in contact with surface waters located downstream of WWTPs, and control herds that had no direct contact with water in Sweden and Spain. In total, 662 fecal samples and 27 water samples were collected. All samples were phenotypically screened for the presence of ESBL- and carbapenemase-producing Enterobacteriaceae, and a randomly picked indicator E. coli isolate was collected from each sample. All the collected isolates were sequenced on a NovaSeq X platform (Illumina) with 2x150 bp. The samples were also screened for 48 genes encoding AMR using smartchip qPCR analysis. We compared the resistance profiles and phylogenetic relationship between the isolates found in cattle samples and those found in water.

Findings: Results from both countries indicate low levels of resistance in cattle, and no differences between control herds and those in contact with water. Additionally, the results indicate that there are no differences in the overall levels of resistance in cattle between the two countries, despite a higher use of antibiotics in Spain.

Implications: The results from this study suggest that contact with waterbodies affected by the effluents of wastewater treatment plants does not act as a driver for the acquisition or emergence of antibiotic resistance in cattle.

Author: María Rincón, Swedish Veterinary Agency (SVA) and Linköping University, Sweden

Co-Authors: Söderlund, Robert; Rosendal, Thomas; Bonnedahl, Jonas; Woksepp, Hanna; Börjesson, Stefan; Hernández, Jorge; Green, Andrew J.; Martín-Vélez, Víctor; Navarro-Ramos, María José

Back to the Program

Session 19

Abstract 204

Room 4

Hosts of antibiotic-resistance genes from Croatian wastewaters

Background and Aim: Wastewater treatment plants (WWTPs) are suitable environments for the interaction of antibiotic resistant bacteria and antibiotic resistance genes (ARGs) and can serve as hotspots of horizontal gene transfer. Influent and effluent wastewater from two WWTPs in Croatia were studied to identify bacterial hosts of clinically significant beta-lactamase genes (blaTEM, blaVIM, blaOXA-48-like) and detect how their composition changes in the treatment process.

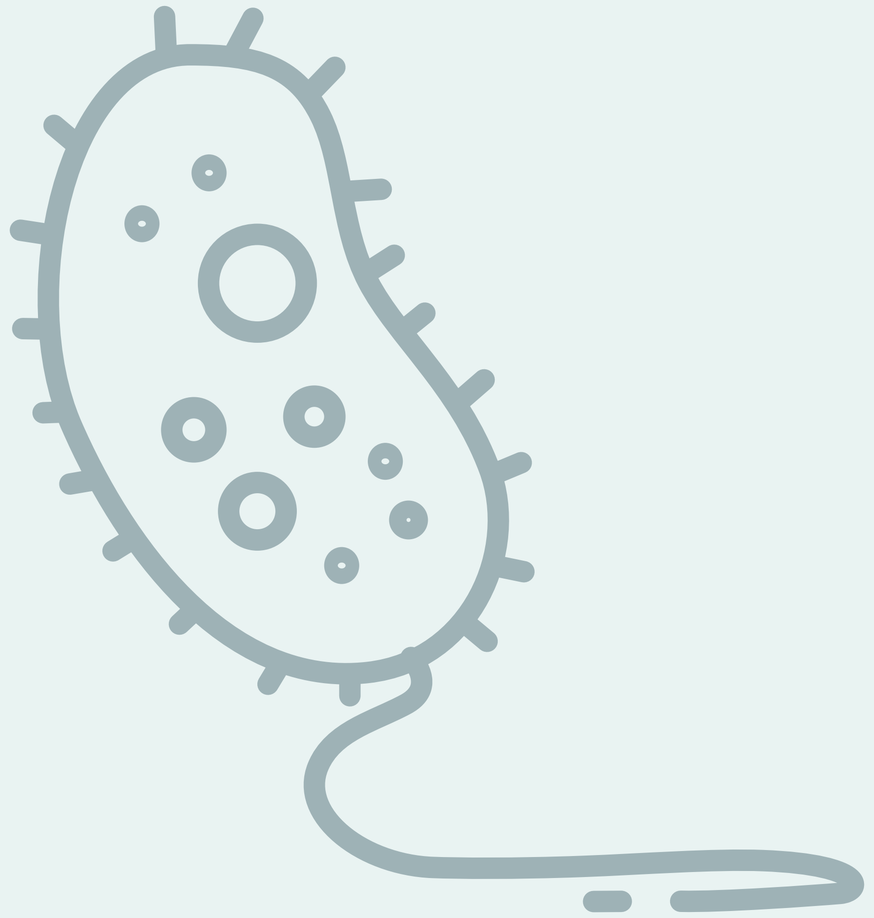
Methods: EpicPCR was used to identify hosts of ARGs in a culture-independent way by amplifying the target gene and the 16S rRNA gene of the host. Additionally, the entire bacterial community was investigated with 16S rRNA amplicon sequencing.

Results: In total, 167 bacterial genera were detected with epicPCR. The blaVIM and blaOXA-48-like genes had a broader host range than blaTEM. Gene blaOXA-48-like was detected in 106 genera, making it the gene with the highest number of hosts in this study. Fourteen clinically significant genera were detected as hosts of the investigated genes with blaOXA-48-like present in all of them. Genus Arcobacter, harbouring all investigated ARGs, was dominant in both WWTPs. Furthermore, clinically significant genera Acinetobacter and Aeromonas harboured all investigated ARGs. Wastewater treatment reduced the number of hosts carrying investigated ARGs. In addition, there were several genera that carried studied ARGs only in effluent, which could indicate possible horizontal gene transfer events.

Implications: EpicPCR provided valuable insights into the bacterial hosts that could serve as potential reservoirs of horizontally transmissible beta-lactamase genes in Croatian wastewater. The methods used in this research provide a good basis for further research aimed at cultivation of specific genera.

Author: Svjetlana Dekic Rozman, University of Helsinki, Finland

Co-Authors: Puljko, Ana; Karkman, Antti; Virta, Marko; Udikovic-Kolic, Nikolina



Back to the Program

Session 20
Abstract 435
Room 3

Investigating AMR evolution in the environment

Large volumes of antimicrobial drugs enter the environment through human, livestock and industrial waste. These waste streams also contain AMR bacteria originating in human and livestock microbiomes, in some cases resulting in correlation between AMR prevalence and antimicrobial residues in receiving environments. This complicates understanding of when, where and under what conditions selection occurs, because bacteria and resistance genes move between microbiomes with very different selective pressures. This presentation will provide an overview of the approaches used to study AMR evolution at environmental antimicrobial concentrations, focusing on methods that can be used to determine AMR dynamics as a function of antimicrobial concentration. Some are routed in ecotoxicology and environmental risk assessment and others in the fields of experimental evolution and inferences from metagenomics and related analyses. Different approaches are necessary to disentangle the complexity of AMR evolution, including: defining selective thresholds or predicted no effect concentrations (PNECs) of antimicrobials, the effect of complex microbial community interactions on selection, the relative strength of selection across antimicrobial concentration gradients, the impact of environmental variables such as temperature, nutrient and oxygen availability on selection, complex mixture effects and the co-selective potential of non antibiotic antimicrobials and other bioactive compounds.

Author: William Gaze, University of Exeter, United Kingdom

Biography:

Professor Will Gaze, Dr Aimee Murray and Dr Anne Leonard lead a large research group within the University of Exeter Medical School's Centre for Environment and Human Health focusing on AMR evolution, ecology and epidemiology in human, animal and environmental microbiomes. WG has worked on the environmental dimension of AMR for >20 years. A particular interest focuses on communicating this subject to non-specialists and developing ideas around One Health and Planetary Health that relate to AMR. There is still a fundamental misunderstanding relating to the role of the environment in the AMR pandemic, and improving communications and developing more effective narratives is likely to improve engagement of cross-sectoral actors. WG has worked with UNEP, writing the first report on AMR and environment which was launched at UNEA 3 in 2017. He also co-authored the EFSA opinion on AMR in food producing environments published in 2021 and has advised WHO, FAO, DG Sante, European Environment Agency, UK and other governments on the environmental dimension of AMR.

Session 20
Abstract 290
Room 3

Effect of pharmaceutical manufacturing waste on the environmental resistome: are there implications for human health?

Background and Aim: Natural environments are affected by several sources of pollution that can contribute to antimicrobial resistance (AMR) selection and spreading. Untreated sewage is recognised as one of the major contributors, while untreated pharmaceutical manufacturing waste is often neglected as a source of environmental antimicrobial pollution contributing to AMR. In this study, we selected a pharmaceutical manufacturing hub in India, Baddi and a control area, Kangra, to study the effects of the pharmaceutical manufacturing waste on the environmental resistome and exposure risks for the local community.

Procedure/Method: Environmental samples (river water/sediment), drinking water and sewage were collected across the areas during wet and dry seasons (2021-2023). Chemical analysis (Active Pharmaceutical Ingredients –APIs– and heavy metals) and molecular analysis by HT-qPCR (42 ARGs, 11 MGEs) and metagenome sequencing were performed to determine antimicrobial and ARG pollution levels and correlations.

Findings/Results: Although seasonal variation in detection of APIs was observed in river water samples, waste released from pharmaceutical manufacturing was detected. Since the pilot study performed in 2019 where high antibiotic concentrations (up to 50 mg/L) were observed in waste stream environmental samples, changes in manufacturing and/or waste disposal led to a decrease in API contamination with antimicrobial concentrations observed being in the ug/L scale.

Waste stream samples where more APIs were detected also correlated to higher prevalence of ARG and MGE markers linked to anthropogenic pollution. In particular, selection of clinically relevant ARGs in environmental samples was observed in the more polluted site.

Implications/Applications: Risk models are being developed integrating the environmental data with faecal resistome and social science datasets collected through interviews with selected stakeholders. These will inform potential routes of exposure of local population to pharmaceutical manufacturing waste and evaluate its impact on the environmental and human resistome to promote policy changes to help mitigate AMR.

Author: Chiara Borsetto, University of Warwick, United Kingdom

Co-Authors: Travis, Emma; Raguideau, Sebastien; Bhatt, Arpita; Maharana, Bhumika; Dafale, Nishant; Quince, Christopher; Song, Lijiang; Taneja, Neelam; Wellington, Elizabeth

Back to the Program

Session 20

Abstract 167

Room 3

Evolution of antibiotic resistance in soils in presence of increased concentrations of sulfamethoxazole and clarithromycin

To face water scarcity, the reuse of treated wastewater (TWW) appears to be a fair and sustainable solution for irrigation of agro-ecosystems. However, TWW can modify soil endogenous communities with the supply of TWW exogenous bacteria, antibiotic resistance genes, and antibiotics likely to induce a selection pressure favouring the dissemination of antimicrobial resistances. Furthermore, due to the low biodegradability of some antibiotics, they can accumulate and reach critical concentrations in soils. To determine a threshold equivalent to the PNEC for soils, increasing antibiotic concentrations in TWW were applied to soil microcosms (0, 10, 100, 500 and 1000 microg/kgdry soil). To do this, sulfamethoxazole (SMX) and clarithromycin (CLA), were chosen.

For SMX, most of the effects were observed within the first incubation week. Several concentration thresholds were established. (i) Between 100-500microg/kgdry soil, a 1st threshold was identified, above which DT50 decreased by a factor of 5. (ii) From 10-100microg/kgdry soil, bacterial selection and inhibition were observed. (iii) A positive correlation between the sul1 and intl1 gene abundance and the SMX concentration was identified. Three levels of resistance were noted within the 1st week according to SMX concentration: 0-10microg/kgdry soil, 100-500microg/kgdry soil, 1000microg/kgdry soil. After 1.5 months, these two genes still exhibited a higher number of copies for SMX concentration above 10-100microg/kgdry soil (+0.5-1 log) suggesting resistance transfer.

For CLA, the first results pointed out a much slower degradation (DT50 > 30 days). We can therefore expect stronger effects on the associated resistance genes and a long-term disturbance of soil microbial communities. The results are currently being analysed.

These experiments enabled to gain a better understanding of the dynamics related to the inputs of these antibiotics and to identify a key SMX concentration between 10 and 100microg/kgdry soil which disturbs soil endogenous communities and whose residual effects are still noticeable after 1.5 months.

Author: Oriane Della-Negra, INRAE, France

Co-Authors: Vittoria Barbieri, Maria; Camotti Bastos, Marilia; Bru-Adan, Valérie; Santa-Catalina, Gaëlle; Ait Mouheb, Nassim; Heran, Marc; Wéry, Nathalie; Chiron, Serge; Patureau, Dominique

Session 20

Abstract 279

Room 3

European Union regulatory measures for the reduction of antimicrobial resistance in the environment - Oversight on the implementation of EU RL 2019/6

Background and Aim: Combating antimicrobial resistance (AMR) is one of the greatest global challenges of our time which can only be addressed through the One Health approach. AMR cannot be adequately addressed by one country alone or be confined to one geographical region. The European Action Plan on AMR addresses these issues and aims to make the European Union a best practice region. The new European legislation on veterinary medicines (Regulation (EU) 2019/6), which applies since January 2022, strengthens the EU's action on AMR by ensuring the prudent and responsible use of antimicrobials in animals and improving data on sales and use of antimicrobials in animals.

Procedure/Method: For the first time, Regulation (EU) 2019/6 establishes directly applicable, harmonized rules for veterinary medicines in all EU Member States. It regulates not only the authorisation and marketing of veterinary medicinal products, but also the use of antimicrobials in animals.

Findings/Results: Concrete measures to combat antimicrobial resistance based on Regulation (EU) 2019/6 include increased requirements for the authorization of antimicrobial veterinary medicines, the restriction of the use of antibiotics for metaphylactic purposes and limitation of prophylactic use to individual animals. In addition, certain antimicrobials have been designated as reserved for the treatment of certain infections in humans, now prohibited for use in animals. In addition, antimicrobials have been defined that may not be prescribed off-label or only be prescribed off-label under certain conditions. To monitor the use of antibiotics in animals, Member States are obliged to collect both sales and use data. Use data collection is performed in a tiered approach starting with food-producing animals in 2023 and ending with non-food producing species in 2030.

Implications/Applications: This overview presents current actions, measures and achievements to combat antimicrobial resistance in veterinary medicine in Europe based on the new European veterinary regulatory framework.

Author: Thomas Heberer, BVL - Federal Office of Consumer Protection and Food Safety -, Germany

Co-Author: Sander, Svenja

Thursday, May 30 - 13:45-15:30

Back to the Program

Session 20

Abstract 126

Room 3

Antibiotic Manufacturing Standard: An industry approach for responsible effluent management in the context of antibacterial resistance

Background and Aim: In the proposal for a new Directive for Pharmaceuticals in the European Union, the scope of the Environmental Risk Assessment has been extended to cover the risk of AMR selection during the lifecycle of antimicrobials, including manufacturing. Moreover, the definition for antimicrobials refers to antibiotics, antivirals and antifungals. However, without a standardized method for the derivation of resistance based PNECs for all antimicrobials, we believe a robust regulatory evaluation of AMR risks cannot currently be conducted in a scientifically reliable way.

On June 14, 2022, the AMR Industry Alliance (AMRIA) published its Antibiotic Manufacturing Standard: Minimizing risk of developing antibiotic resistance and aquatic ecotoxicity in the environment resulting from the manufacturing of human antibiotics. The Standard, facilitated by the British Standards Institution (BSI), provides clear guidance to manufacturers in the global antibiotic supply chain to ensure that their antibiotics are made responsibly, helping to minimize the risk of AMR in the environment.

Procedure/Method: The Antibiotic Manufacturing Standard includes science-based PNEC targets for risk assessments to effectively control antibiotic releases from manufacturing operations. It requires an environmental management system and risk-based approach to assessing and controlling antibiotic manufacturing waste streams, and adherence to the Alliance's published PNECs.

Findings/Results: Provision of clear guidance and an assurance mechanism for manufacturers, builds global awareness, encourages decisive action, and provides a new model of transparency for the industry.

Implications/Applications: Inclusion of the Standard in antibiotic tenders is currently being considered (for example in Nordic Countries) to recognize the importance of AMR risk reduction in antibiotic production. The Standard can also serve as a model to assess antibiotics in upcoming legal implementations.

Author: Andreas Häner, F. Hoffmann-La Roche Ltd, Switzerland

Co-Authors: Elmoznino, Joanne; Reid, Kirsty; Brooks, Steve

Friday, May 31 - 8:30-10:15

Back to the Program

Session 21

Abstract 420

Room 3-4

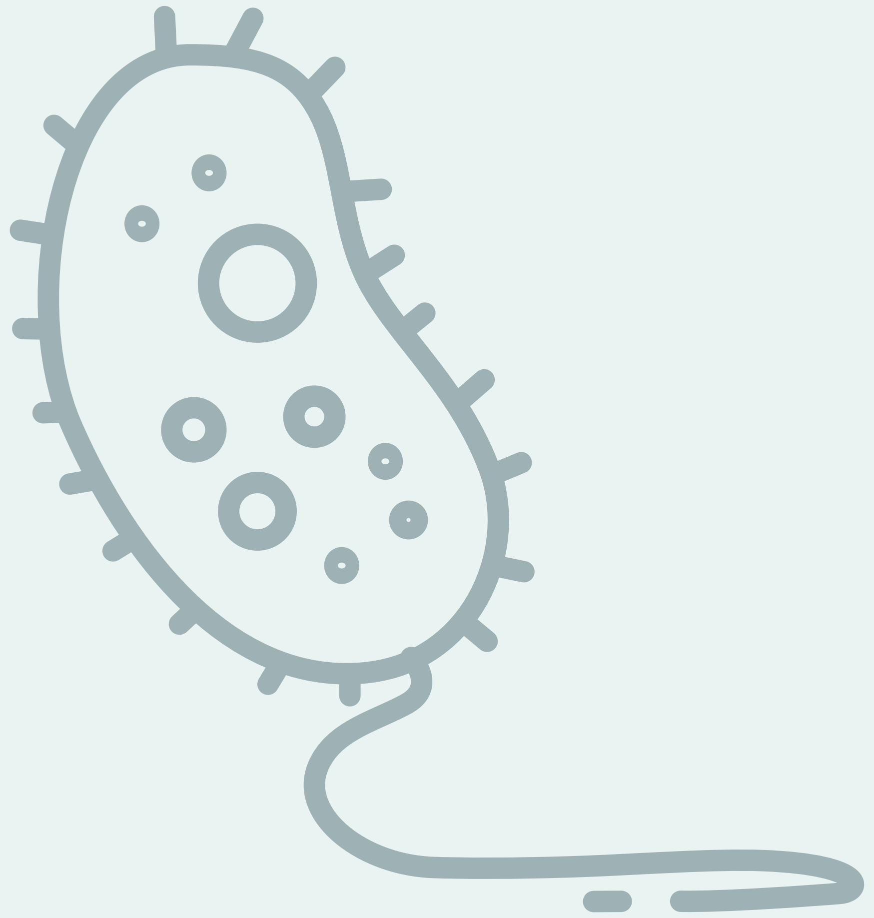
Taking a Systems Approach to Addressing the Environmental Dimensions of Antimicrobial Resistance

In 2023, the UN Environment Program's report, Bracing for Superbugs, placed a spotlight on the environmental dimensions of antimicrobial resistance (AMR). One of the key challenges was in translating the science into actionable policy recommendations. Bridging this gap, the report traced the key drivers of AMR's spread in the environment through three economic sectors and their value chains—pharmaceutical manufacturing, agri-food systems, and healthcare delivery—as well as municipal sewage and waste systems. This placed SDG12 (Responsible consumption and production) more centrally into a systems approach to tackling AMR. Averting the consequences of AMR meant ensuring that consumption and production of antimicrobials occurred within the carrying capacity of these value chains and the ecosystem. Complicated by globalization and urbanization, how might research addressing the emergence of AMR and hotspots of drug-resistant infections be prioritized and support the global response in the context of these value chains and other development efforts?

Author: Anthony So, Johns Hopkins Bloomberg School of Public Health, United States

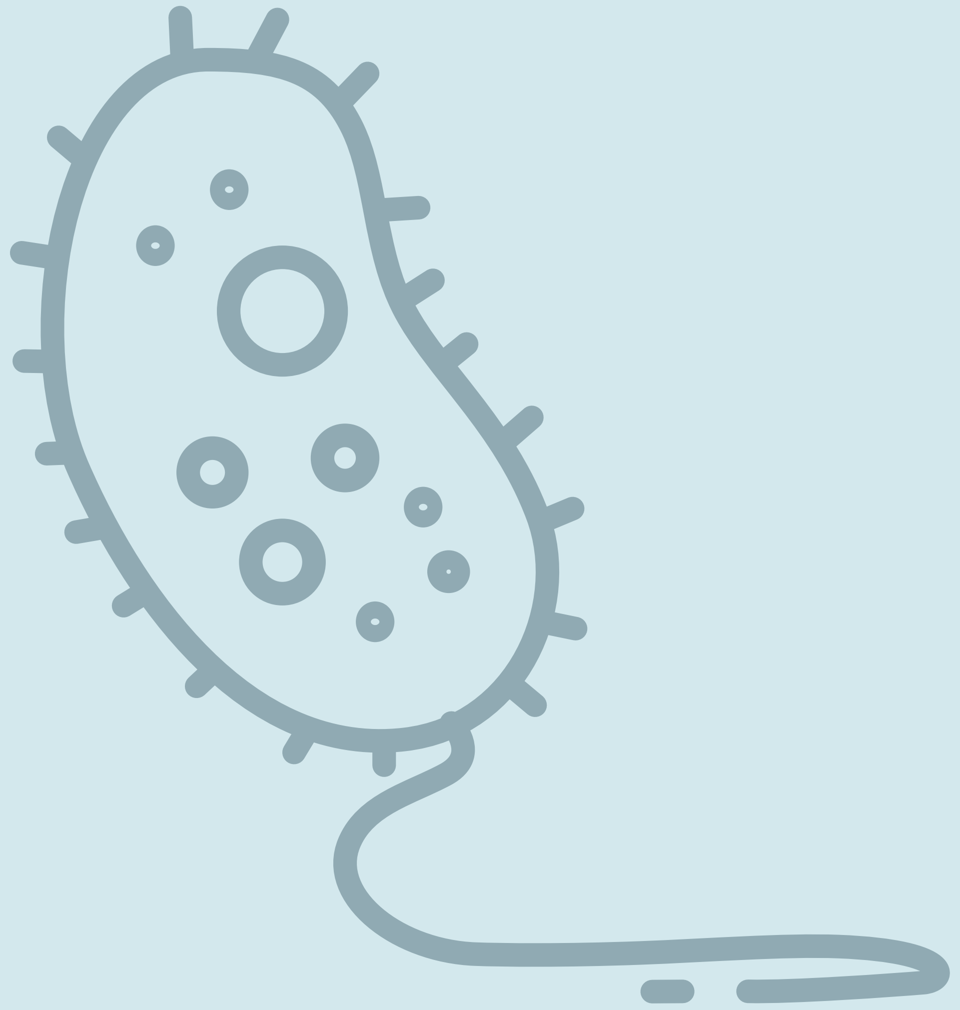
Biography:

Anthony D. So, MD, MPA is a Distinguished Professor of the Practice at the Johns Hopkins Bloomberg School of Public Health and the Founding Director of the Innovation + Design Enabling Access (IDEA) Initiative in the Department of International Health. The Initiative also serves as the Secretariat for the Antibiotic Resistance Coalition (ARC), an alliance with over twenty civil society groups and the South Centre. Professor So has served as Co-Convener of the UN Interagency Coordination Group on Antimicrobial Resistance; was part of the Antibiotic Resistance Working Group of the U.S. President's Council of Advisors in Science and Technology; and conducted research on reengineering how antibiotics are brought to market as a recipient of the Robert Wood Johnson Foundation Investigator Award in Health Policy Research. He was a co-lead author of the UN Environment Program's global spotlight report on the environmental dimensions of AMR in 2023.



Poster Session A

1 - Impact of wastewater on the invasion of antibiotic resistant bacteria in river biofilms
Irina Dielacher
3 - Impact of temperature, feedstock, and seasonality on antibiotic resistance genes in hyper-mesophilic anaerobic digestion of agricultural biomass
Bing Guo
4 - Large-scale screening of antimicrobial resistance genes along 2300 km of the Danube River
Alexander Kirschner
5 - Antibiotic resistome of final effluents from wastewater treatment plants across China
Li-Juan Li
6 - Antimicrobial Resistance in the Adyar River, India, and its Control by Electro-Oxidation
THARA M V
7 - Linking Antimicrobial Resistance Dissemination to Human Fecal Pollution: A Study on CrAssphage and ARGs in Hospital Wastewater and Environment
Prasert Makkaew
8 - Livestock farm air is a critical pathway in disseminating high-risk antibiotic resistance genes
Xun Qian
9 - Carbapenem-resistant Pseudomonas aeruginosa in hospital wastewater and the water chain in Indonesia and the Netherlands
Selvi Nafisa Shahab
10 - Dynamics of Extracellular Antimicrobial Resistome in Full-scale Membrane Bioreactor and Conventional Activated Sludge Processes
Mardalisa Mardalisa
11 - Sub-minimum inhibitory concentrations of florfenicol and tetracycline enhanced horizontal transfer of AMR genes between Salmonella strains
Amer Abdelgany
13 - The alteration of microbial biodiversity in the receiving environment promotes its invasion by anthropogenic antibiotic resistance genes
Christophe Merlin
14 - Antibiotic resistance dimension in Belo Horizonte, Brazil, through the lens of hospitals and communities wastewaters
Juliana Calábria de Araújo
15 - Tracking ARGs through a Potable Water Reuse Treatment Train
Matthew Blair
17 - Key Elements within an Integrated Monitoring Framework of Antimicrobial Resistance for Asian Countries
Ryo Honda
18 - Investigation of antimicrobial resistance in sewage and effluent from stabilization lagoons in the Belo Horizonte region, during the COVID-19 pandemic
Juliana Calábria de Araújo
19 - Removal of antibiotic resistance genes in full-scale wastewater treatment plants using aerobic and anaerobic systems in Brazil
Juliana Calábria de Araújo
20 - Development of Copper Resistance in Legionella pneumophila via Experimental Evolution
Gillian Cameron
21 - Loss of microbial diversity and abundance of resistomes during termite mound formation
Qinglin Chen
22 - Below the surface: The hidden consequences of eutrophication on river resistome, mobilome and virulome
Mira Cooper-Beknazarova
24 - Clinical class-one integron recombination is linked to prokaryotic stress response during conventional biological wastewater treatment
Jay Garland



Back to the Program

Poster Session A

25 - Impact of simple versus complex wastewater compositions on the fate of antimicrobial resistance genes in activated sludge wastewater treatment
Emmanuel Diaz Mendoza

28 - 'Resistome' of Kettle Lake in Ontario, Canada Includes 16S and 23S rRNA Mutations Conferring Antimicrobial Resistance
Anastasia Fedynak

29 - Antibiotic resistome beneath the surface: River biofilms reveal selection pressure of environmentally relevant concentrations of antibiotics.
Gabriela Flores-Vargas

30 - Antimicrobial resistance dissemination in hospital wastewater and associated aquatic environment in Joinville, Southern Brazil: A One Health approach
Paulo Franca

31 - Antimicrobial resistance and antimicrobial residues in the different stages of commercial poultry environment and across the food chain
Pankaj Gaonkar

32 - The Wastewater Resistome: a 29-month survey of Quebec City wastewater metagenomes reveals plasmids as drivers of emergent antimicrobial resistance
Jeff Gauthier

33 - Characterisation of antimicrobial-resistant Escherichia coli isolated from retail chicken breasts
Paul Glenn

34 - Understanding roles of non-antibiotic pharmaceuticals on the emergence and transmission of antibiotic resistance
Zhigang Yu

35 - Chlorination stress can promote horizontal transfer of antibiotic resistance genes within mixed-culture biofilms
Zhigang Yu

36 - A nanopore long-read sequencing points out a modification of hospital wastewater microbiome and resistome after chlorine disinfection
Monika Harnisz

37 - Fate of antibiotic resistance genes and mobile genetic elements during mesophilic anaerobic digestion and post treatment of digestate in french farms.
Alain Hartmann

38 - The selective effects of commonly used non-antibiotic pharmaceuticals in combination with ciprofloxacin
April Hayes

39 - Tracking antibiotic resistome structure and its driving factors from Qiantang River Basin to the downstream Hangzhou Bay
Xinyu Huang

40 - Antimicrobial Resistance in Wild Escherichia coli Isolates from Whitetail Deer
Asalia Ibrahim

41 - The impact of nitrogen fertilization on antimicrobial resistance genes in Rhizobium leguminosarm trifoli.
Isabella Ippolito

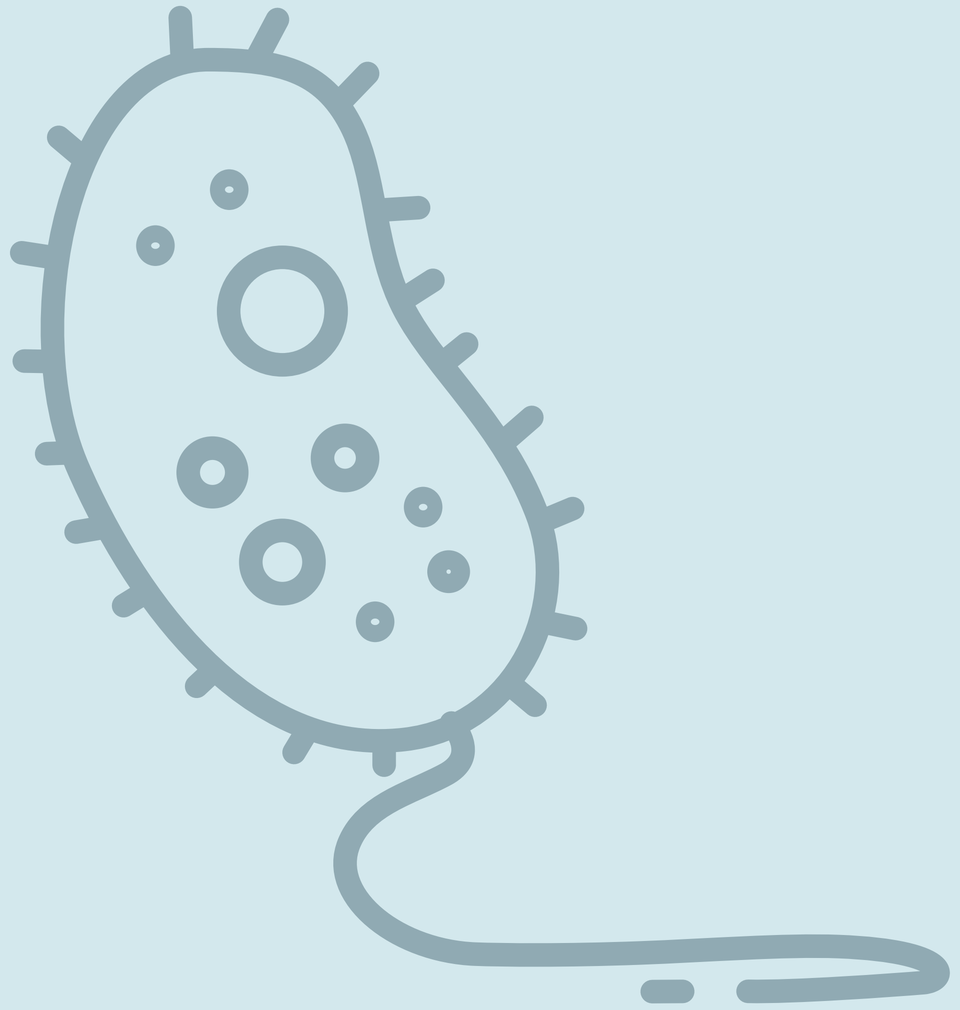
42 - Mobility potential of latent antibiotic resistance genes
Antti Karkman

43 - Optimizing DNA extraction and sequencing approach for recovering ARG contextual information in wastewater
Chad Kelley

44 - Trimethoprim resistance in surface and wastewater is mediated by contrasting variants of the dfrB gene
David Kneis

45 - Human and veterinary antibiotics use drives resistome diversity across hundreds of lakes
Susanne Kraemer

47 - Abundance of antimicrobial resistance genes in clinically relevant bacterial pathogens isolated from a recreational freshwater lake in Ontario, Canada
Opeyemi Lawal



Back to the Program

Poster Session A

49 - Cumulative effects of sub-inhibitory erythromycin on soil antibiotic resistance genes

Ziming Han

50 - Transmission of antibiotic resistance in a model soil detritus food chain under pesticide stress

Zhelun Liu

52 - Xenogenetic Evolutionary of Integrons Promotes the Environmental Pollution of Antibiotic Resistance Genes

Liping Ma

53 - Bacteriophage-associated antibiotic resistance genes are abundant before but not after wastewater treatment

Goncalo Macedo

54 - Impact of Wastewater Effluent on Surface Waters Resistomes in a Low-Income Community with Failing Sewage Infrastructure

Gabriel Maldonado Rivera

55 - Fate of antibiotic resistance and organic micropollutants in small-scale wetland-based municipal treatment systems

Celia Manaia

56 - Resistance against last-resort antibiotics in pathogens and novel resistance factors are prevalent in sewage from Norway

Nachiket Marathe

57 - The AMR challenge: extended-spectrum beta-lactamase-producing Enterobacteriaceae in two anthropogenically distinct areas in Uganda

Charles Masembe

58 - Impacts of chemicals and microbiota from hospitals on the emergence of resistance in sewer systems

Sarah Naudin

59 - Metagenomic comparison of wastewater treatment plant effluent and other putative sources of AMR to surface water in urban Mozambique

Loc Nguyen

60 - Dissemination of antimicrobial resistance (AMR) upstream and downstream of a wastewater treatment plant in rural Southwest Virginia, USA.

Idowu Okeshola

64 - Hospital effluent is as a source for novel antibiotic resistance genes reaching the marine environment in Norway

Vera Radisic

65 - Sequencing of ESBL E. coli from manure across four countries reveals high prevalence in all regions but distinct resistance patterns.

Nicole Ricker

66 - Natural Transformation is affected by sub-lethal concentration of antibiotics in freshwater bodies conditions.

Francesco Riva

67 - Seasonal impact of the metropolitan wastewater treatment plant on the resistome and plasmidome of sediments in the Vistula river

Anna Rokowska

68 - Safer, fit-for-purpose wastewater treatment: focus on antibiotic resistance

Afonso Ruivo

70 - Bacterial dynamics of the plastisphere microbiome exposed to sub-lethal antibiotic pollution

Concepcion Sanchez-Cid

71 - Effects of Antibiotic use cessation on the soil resistome and microbiome of horse farms: is it enough to curb resistance reservoirs?

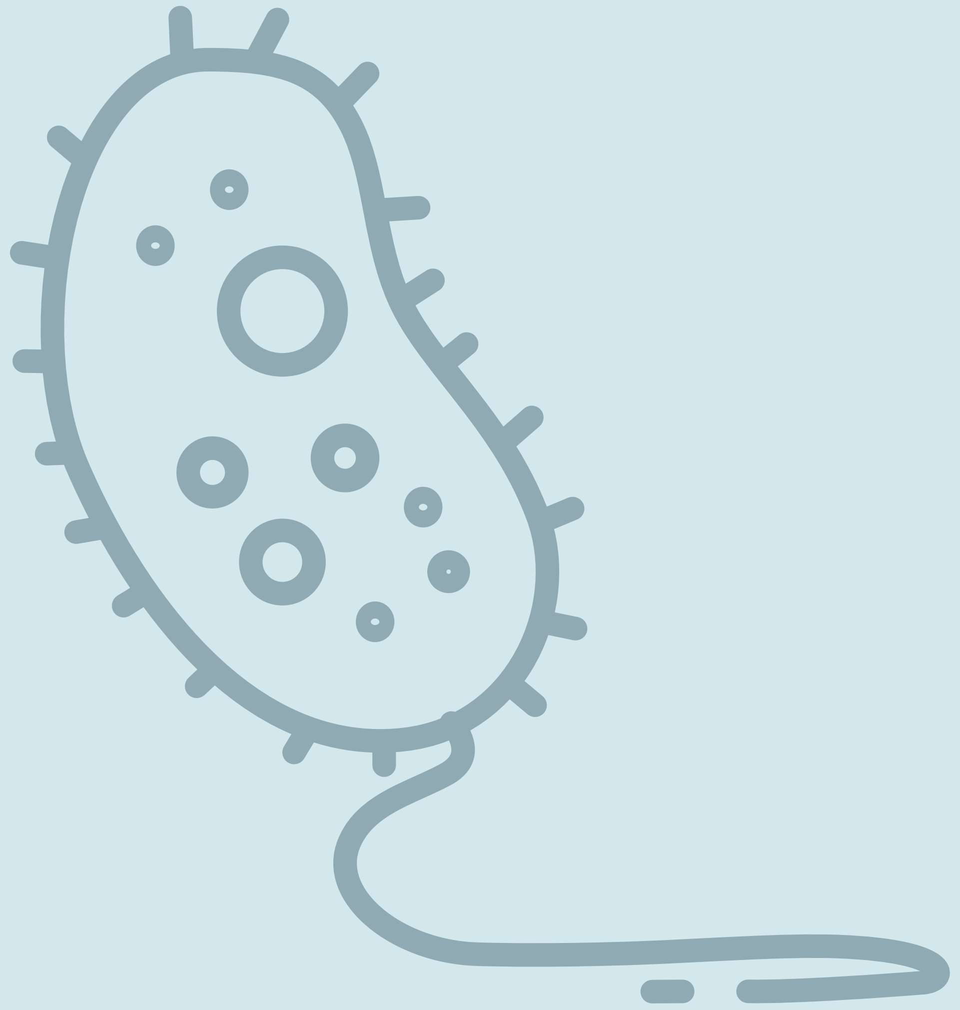
Alinne Santana-Pereira

72 - Dissemination of antibiotic-resistant bacteria in sewage: is there a correlation between bacterias resistant to different antimicrobials?

Juliana Calábria de Araújo

73 - Resistance to critically important antibiotics in meat sold for consumption

Jordan Sealey



Back to the Program

Poster Session A

74 - Evaluation of the implementation of the strategic plan for surveillance and control of antimicrobial resistance 2018-2022 in Senegal.
Ibrahima Mamby KEITA

75 - Antibiotic Resistance Gene Abundance and Transfer in Wastewater-Irrigated Soils
Kornelia Smalla

76 - Assessing the reproducibility of whole-microbial community evolution of AMR: unraveling the contributions of vertical and horizontal gene transfer
Lisa Stabryla

77 - Selective Colonisation of Microplastics by Antimicrobial Resistant and Pathogenic Bacteria
Emily Stevenson

79 - Spatial and temporal spread of antimicrobial resistance in aquatic environments
Máté Vass

80 - Unearthing the dark side of Glyphosate: boosting antimicrobial resistance and virulence in Klebsiella species.
Katie Wall

81 - The overlap of soil and vegetable microbes driving the transfer of antibiotic resistance genes from manure-amended soil to vegetables
Fenghua Wang

82 - Molecular Characterization of Extended-Spectrum β-lactamase-producing Escherichia coli isolated from in a River Basin in Yamagata Prefecture, Japan
Toru Watanabe

83 - Phenomic analyses as means to identify drivers of selection in hospital wastewater
Noel Waters

84 - Specialized metabolite from wheat leaves triggers Pseudomonas-antibiotic resistance under host genetic variations
Qian Xiang

86 - Comparison of genetic traits between cephalosporin resistant Escherichia coli and Klebsiella pneumoniae from human and environmental sources
Qiyi Yuan

87 - Antimicrobial burden across a gradient of anthropogenic impact on the Lower Great Lakes
Arthur Zastepa

88 - The AMR Multi-Stakeholder Partnership Platform: catalyzing a global movement for action against AMR
Paola Grenni

89 - Effects of Ozonation and Post-Treatment Biofiltration on AMR in Swiss Wastewater Treatment Facilities
Helmut Bürgmann

90 - Plant-based Natural Products as Potential Phytobiotics
Ibukun Busari

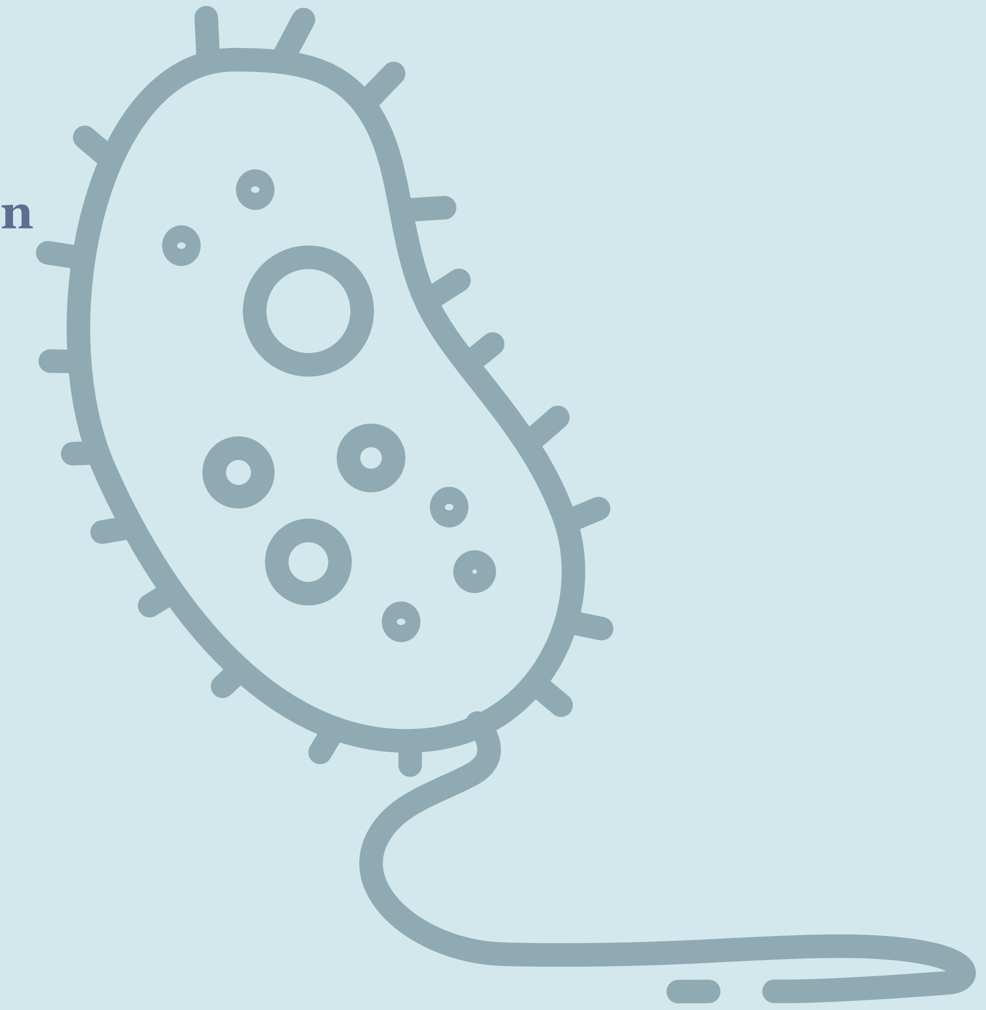
91 - Assessing Antagonistic Interactions of Staphylococcus xylosus and Aerococcus urinaeequi Against Bovine Mastitic Staphylococcus aureus
Zhangbin Cai

92 - ARG removal or intrinsic population selection in anaerobic digestion to limit resistance dissemination? There is the question!
Natalia Klimova

95 - Longitudinal Analysis of Antimicrobial Resistance Gene Dynamics in Wastewater across Four Central Canadian Communities
Jade Daigle

96 - The influence of in-situ pilot-scale hospital wastewater treatment on antibiotic resistance determinants and total and viable microbial community
Iakovos Iakovides

98 - Use of conifer needles as passive samplers for antibiotic resistance genes (ARGs) in vicinity of swine
Samantha Leclerc



Back to the Program

Poster Session A

99 - Wastewater Chronicles: Comprehensive Analysis of Antibiotic Resistance in Wastewater
Adriana Osinska

101 - Compact decentralized advanced treatment methods to combat the release of AMR in wastewater effluents from AMR point-sources.
Carsten Ulrich Schwermer

106 - Development of pulsed blue light technologies for bacterial biofilm disruption
Violet Vakunse Bumah

108 - Thermophilic ananerobic digestion reduced the abundance but increased the horizontal gene transfer and risk level of antibiotic resistance genes
Junya Zhang

109 - Dirty habits: potential for spread of antibiotic-resistance by black-headed gulls from waste-water treatment plants
Víctor Martín Vélez

112 - Determination of Chemical Pollutants as Drivers for Antimicrobial Resistance in an Agricultural Watershed
Damalka Balasuriya

113 - Antimicrobial-resistance genes in bioaerosols from Livestock operations
Alejandra Castillo Toro

114 - Antibiotic Resistance fate: dead, survive or thrive in the microbial jungle depending on shaping effects of associated microbiota in aquaponics
Frédérique CHANGEY

115 - Application of "under the radar" approaches to identify anthropogenically derived pathogen and AMR indicators in produce and soil
Chagai Davidovich

116 - Can anaerobic digestion reduce the prevalence of antimicrobial resistance genes in UK poultry litter?
Sarah Guest

118 - Effects of antimicrobial use on minimum inhibitory concentrations in Salmonella, Campylobacter and E. coli isolates from poultry.
Laura Huber

121 - Composting reduces the risks of resistome in beef cattle manure at the transcriptional level
Xu Li

122 - The co-occurrence and potential transfer risks of antibiotic resistomes between non-intensive aquaculture environment and human
Yanping Mao

124 - Genomic Comparison of Klebsiella pneumoniae Isolated from Bovine Clinical Mastitis and Human Infections
Bridget O'Brien

125 - Optimizing the Bovine Microbiome to Reduce Agricultural use of Antibiotics
Jennifer Ronholm

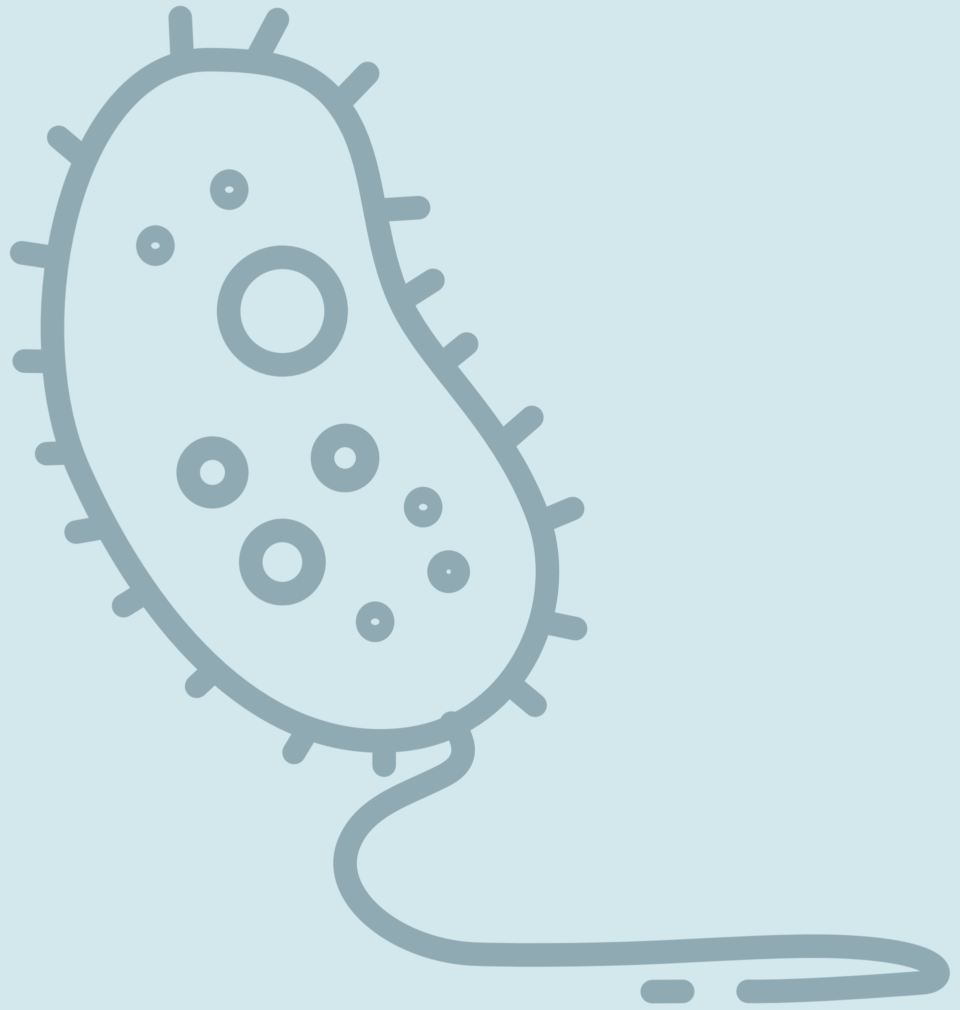
126 - Reduction of resistant bacteria in manure treatment across 4 countries
Heike Schmitt

127 - Impact of soil type on the dissemination of antibiotic resistance genes along the soil-plant continuum
Yaqiong Song

128 - A Systematic Review on Swine Pathogens in China: Trends, Prevalence, and Implications for Public Health and Biosecurity
Thanaporn Thongthum

131 - Hazard reduction and persistence of risk of antibiotic resistance during thermophilic composting of animal waste
Wan-Ying Xie

135 - Turning One Health policy into action: Working with industry to prevent and mitigate AMR
Erica Donner



Back to the Program

Poster Session A

137 - Potential for FLOS LAMP as an Affordable Method for Quantification of Antimicrobial Resistance Indicator Genes in Low-Resource Settings
Petra Choi

138 - Metagenomic tools to detect emerging pathogens and antimicrobial resistance genes for wastewater-based surveillance
Ju-Ling Liu

141 - Phenotypic Metagenomics Reveals Wastewater-Associated Rare but Clinically Important Broad-Spectrum Beta-lactam Resistance Invading River Ecosystem
Guoqing Zhang

142 - Enhanced taxonomic resolution for antibiotic resistance gene host bacteria identification using modified epicPCR
Shihai Liu

146 - Whole-genome sequence of Aeromonas hydrophila CVM861 isolated from diarrhetic neonatal swine
Toni Poole

147 - Molecular epidemiology and mechanisms of carbapenem and colistin resistance in Klebsiella and Enterobacter from treated wastewater
Ana Puljko

149 - Proximity-guided metagenomics to unravel physical association between plasmid-borne antimicrobial resistance genes and hosts in wastewater in Vietnam
My Hanh Vu Thi

151 - Methane and Nitrous Oxide Emissions from Manure: Effects of Antibiotic Residues
Chebet Arusey

153 - Antimicrobial Resistance, Environmental Racism & Climate Change: A Deadly Triangle
Zanah Francis

156 - An analysis of wastewater antimicrobial resistance genes before and after the lifting of COVID-19-related travel restrictions
Jangwoo Lee

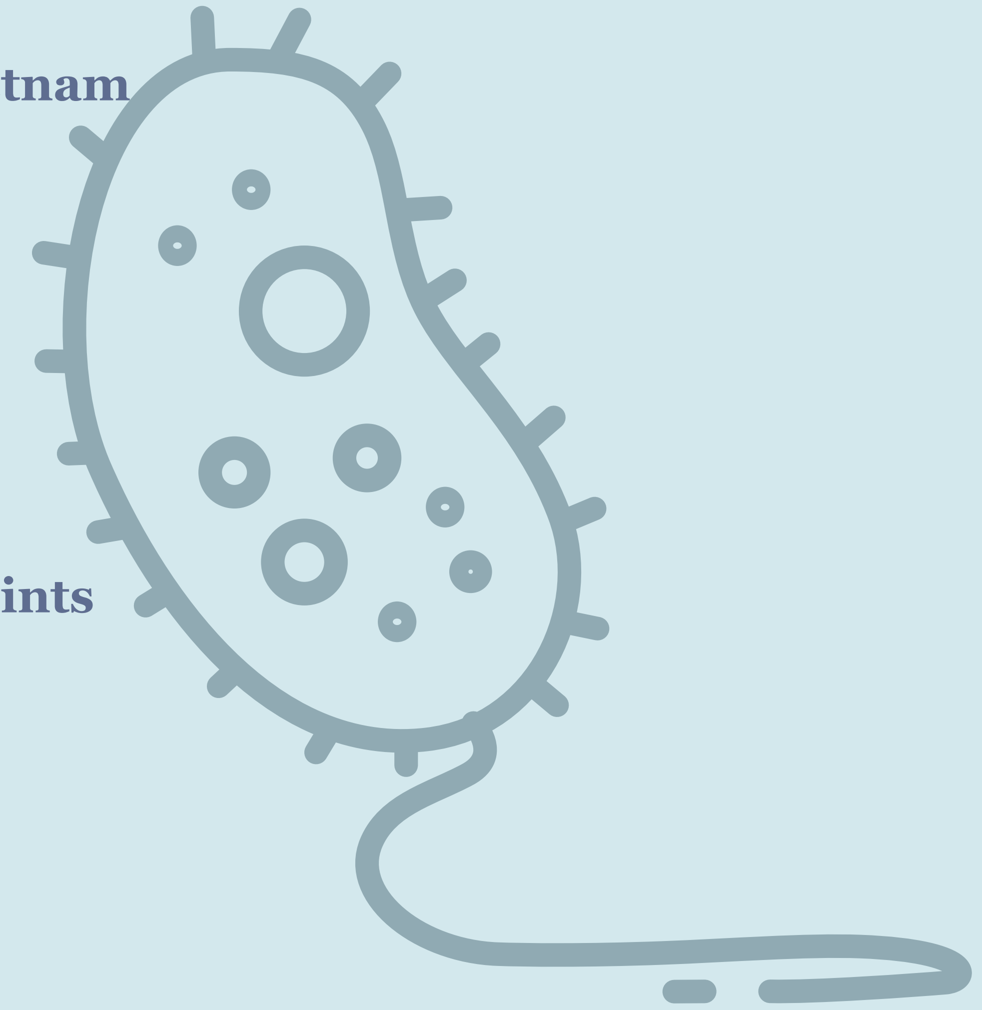
158 - A vision for metagenomic-enabled environmental AMR surveillance that is adaptive to low- and high-income settings and water infrastructure constraints
Peter Vikesland

160 - What about outside the cell? Abundance and diversity of intracellular and extracellular ARGs in urban stormwater and surface waters
Kassidy O'Malley

161 - Microbiome Diversity and Biosafety of Digestate Derived from Agricultural Biogas Plants in Circular Waste Management
Ewa Korzeniewska

162 - Eradication of Staphylococcus aureus Biofilms and Antibiotic-Tolerant Persisters by Enantiomeric Glycosylated Cationic Block Co-Beta-Peptides
Mary Chan-Park

163 - Novel Cationic Antibacterial Polymers as Potential Antibiotic Replacements
Mary Chan-Park



Back to the Program

Poster Session B

1 - Spatiotemporal surveillance of antimicrobial resistance from wastewater treatment plants in rural West Virginia (USA)
Christopher Anderson

4 - Towards the development and evaluation of a sewage surveillance system to predict antibiotic resistance in Klebsiella pneumoniae from humans
Julián Bobis

5 - GPS-tagged Black-headed gulls give insight into the dispersal of Carbapenemase-producing Enterobacterales from WWTP:s
Jonas Bonnedahl

6 - Carbapenemase gene blaOXA-48 detected at six freshwater sites in Northern Ireland discharging onto identified bathing locations.
Cathy Brooks

7 - Comparison of antibiotic resistant bacteria and antibiotic resistance genes abundance in hospital and domestic wastewaters in Brazil
Juliana Calábria de Araújo

8 - Household WASH characteristics and ESBL E. coli in stored drinking water: Analysis in Kenya
Molly Cantrell

9 - Antibiotic Resistome in Sewage Pipeline Connected from Hospital Sewage Effluent to a Wastewater Treatment Plant
Jong-Chan Chae

10 - Enrichment of antibiotic resistant zoonotic Aeromonas veronii and its potential transmission risk to humans and animals
Zeyou Chen

11 - Considerations for Wastewater-Based Epidemiology of AMR in Communities with Compromised Sewage Collection Networks
Amanda Darling

12 - Effects of different wastewater sampling approaches on antibiotic resistance gene determination and other molecular biological analyses
Irina Dielacher

13 - Exploration of antibiotic resistome in Hong Kong communities through wastewater metagenomic surveillance
Jiahui DING

14 - The Australian silver gull plays a role as by-stander host to pathogenic and antimicrobial resistant Escherichia coli
Steven Djordjevic

15 - Sewage surveillance of antibiotic resistance - possibilities, limitations and pitfalls
Carl-Fredrik Flach

16 - Implementing a Surface Water Pilot within a National, One Health Focused Antimicrobial Resistance (AMR) Monitoring System in the United States (U.S.)
Jay Garland

17 - AMR-Ecogenomics: Microbial Ecosystems as Drivers of the Antimicrobial Resistome
Jeff Gauthier

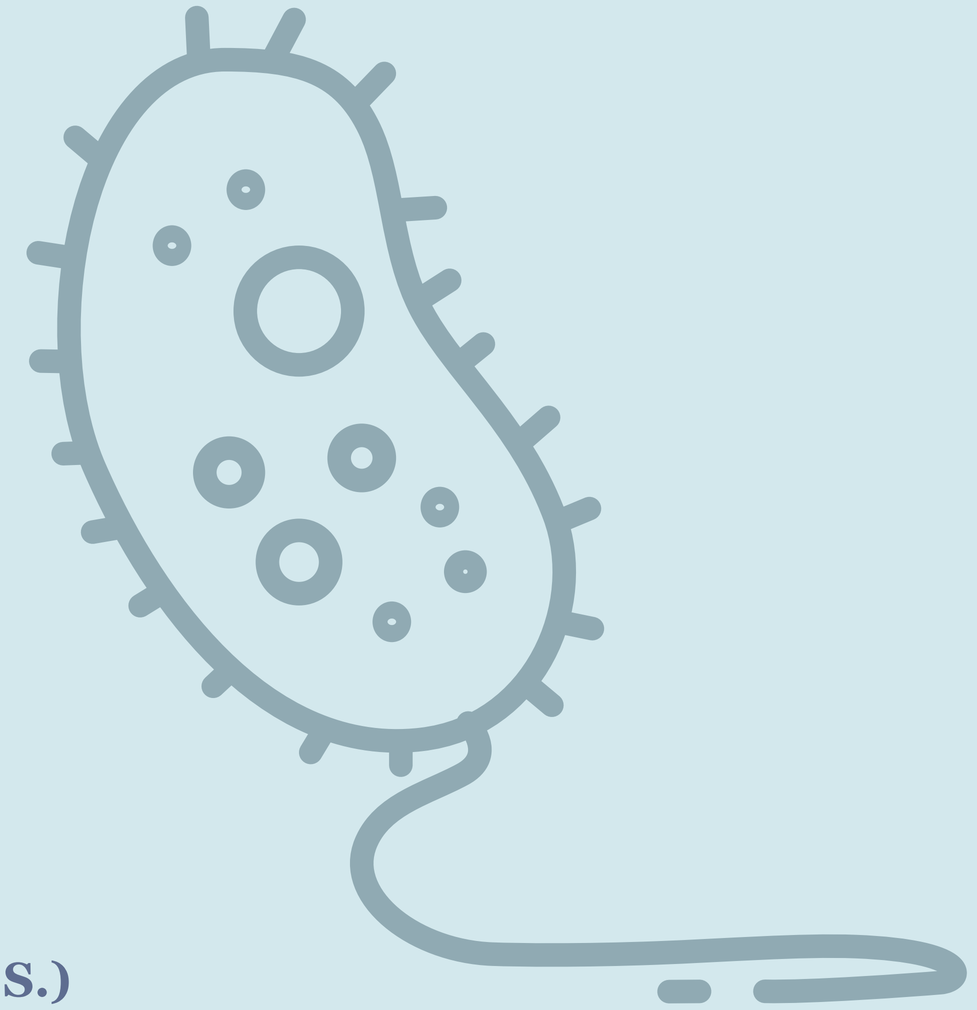
19 - Identifying Antimicrobial Resistance Gene Sequence Variants to Track the Dissemination of Antimicrobial Resistance Between Environmental Reservoirs
Claire Gibson

20 - AMR Transmission and Nutrition in Infants in Informal Settlements – Behaviour vs Environment
David W Graham

21 - Antibiotics and antibiotic resistance genes in an urbanized River
Paola Grenni

22 - Environmental detection of MRSA and VRE in single-patient rooms
Alexandra Hicks

25 - The influence of predation by Tetrahymena borealis on conjugation in Aeromonas salmonicida subsp. salmonicida
Alicia Durocher

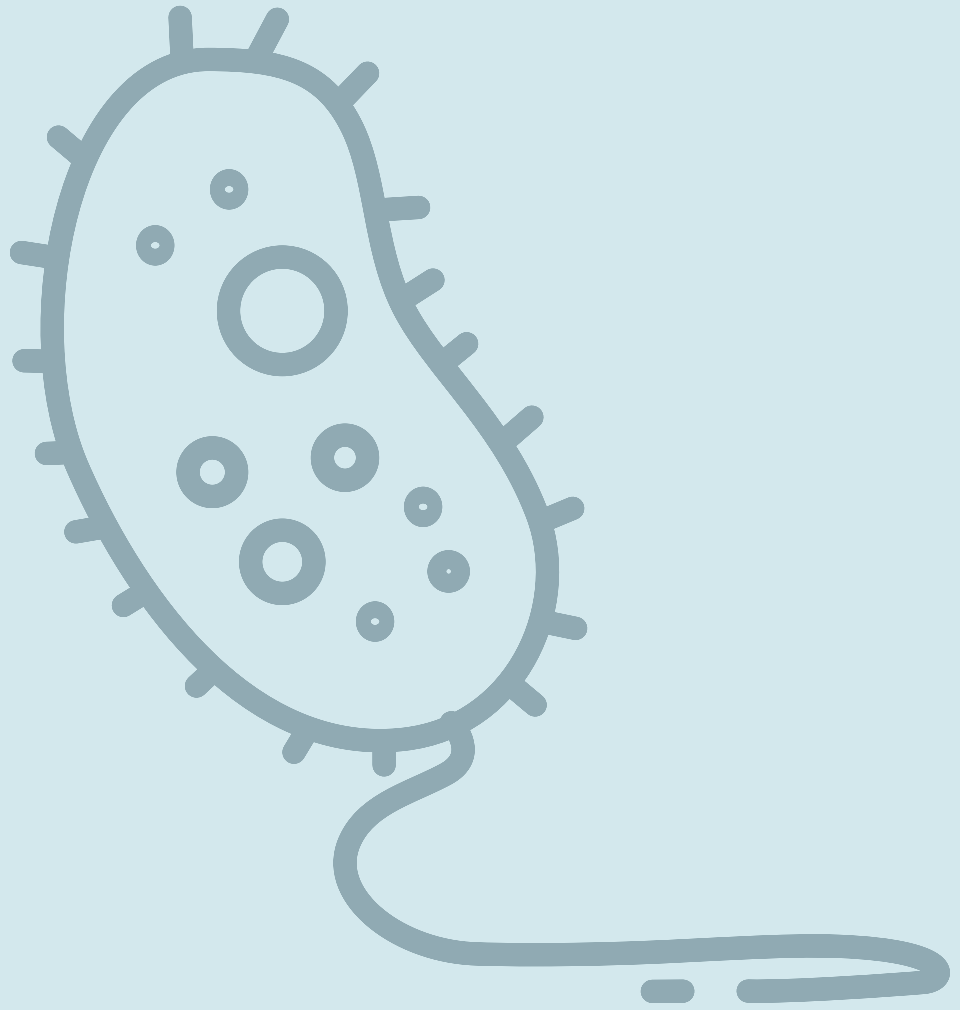


Back to the Program

Poster Session B

Environmental Dimension of Antimicrobial Resistance

26 - Demonstrating integrated wastewater AMR surveillance for city-scale health protection
Kelly Jobling
27 - Development of a NIST Standard Reference Material for Environmental and Clinical Antibiotic Resistance
Ishi Keenum
30 - Biodiversity and Antimicrobial Resistance Genes in Bioaerosols: Results of an Across-Canada Assessment using Car Air Filters
Paul George
31 - Monitoring the potential escape of bioaerosolised antimicrobial resistance genes from wastewater treatment plants with air and phyllosphere sampling
Paul George
32 - Association of population's lifestyles and behaviours with the prevalence of antimicrobial resistance in municipal wastewater
Helena Leal
33 - Barriers and Opportunities for Monitoring AMR in the Environment in LMICs
Emilie Gilles
35 - Deciphering antibiotic resistome, mobilome, and microbiome of river water in Hong Kong by metagenomic sequencing
Xuemei MAO
37 - Impact of Wastewater Discharge on Antibiotic Resistance and Microbial Communities in Fish Guts and Urban Rivers
Molly Mills
39 - Assessing the value of sewage surveillance to predict antibiotic resistance
Gilbert Osena
41 - Metagenomic analysis of antimicrobial resistance genes in municipal wastewater in a two-year longitudinal study in Aotearoa New Zealand
Isabelle Pattis
42 - Current knowledge of AMR in wild fauna and flora
Rachel Payne
43 - Investigating the occurrence of antimicrobial resistance in Canadian environments: A scoping review.
Manuel Perez Maldonado
44 - Comparing the Occurrence of Antibiotic Resistance Genes in Septage and Wastewater Influent in Southwest Virginia
Sarah Price
45 - Hydrodynamic model-assisted tracking of antimicrobial resistance distribution in Lake Geneva
Sasikaladevi Rathinavelu
49 - Impacts of Antibiotic Pesticide Use in Crop Agriculture on Antibiotic Resistance in Human Pathogens and Commensals: A Systematic Review
Mary Schoen
50 - Evaluating a metagenomic workflow for nontargeted detection and quantification of AMR and disease agents in wastewater
Katherine Scott
51 - Bioaerosols in Arid Climates: A One Health Approach to Antibiotic Resistance and Viral Surveillance in Qata
Azhar Siddique
52 - Antibiotic Resistance Genes in Wastewater and Environmental Water and Implications for Thailand National Action Plan on Antimicrobial Resistance
Kwanrawee Sirikanchana
53 - Small river meets big city: Sewage pollution drives AMR and detectable through simple proxies
Vikas Sonkar
54 - Differential Antibiotic Resistance Profiles in Wastewater and Public Spaces Across Varied Housing Densities in Makassar, Indonesia
Sulfikar Sulfikar



Back to the Program

Poster Session B

55 - GastroPak : Tracking sources of gastroenteritis in Pakistan
Kim Summers

57 - Challenges of outdoor air sampling for AMR surveillance
Nathalie Turgeon

58 - Monitoring antibiotic resistance genes in Austrian water systems and exploring links with (environmental) parameters
Julia Vierheilig

59 - Investigation of Antibiotic-Resistant Escherichia coli Prevalence in Red Fox in a Rural Area of Northern Japan Using Culture-Method
Masateru Nishiyama

60 - Guide to strengthen monitoring and surveillance of AMR in farm animal environment: fostering a one health approach to integrated AMR surveillance
Muhammad Usman Zaheer

61 - Fate and Control of Clinically Relevant High-risk ARGs in Livestock-Related Environment
Ziming Han

62 - Pharmaceuticals at concentrations exceeding PNEC values in the aquatic environment fail to induce antimicrobial resistance in Escherichia coli
Luther King Abia Akebe

63 - Soil colloids affect the selective effect of different antibiotics on bacterial growth
Katharina Axtmann

64 - Distribution of antibiotic resistance between phages and bacteria in a river ecosystem receiving treated wastewater
Xavier Bellanger

65 - River Biofilms and AMR – Insights from Field and Laboratory Studies in Switzerland
Helmut Bürgmann

66 - A Framework for Modeling Microbial Ecological Alterations of the Resistome in the Sewershed
Thomas Byrne

67 - The Minimum Ciprofloxacin Concentration Affecting the Microbial Community and Antimicrobial Resistance Gene Compositions of Anaerobic Processes
Sukriye Celikkol

68 - Community succession and antibiotic resistance in river biofilms on natural and artificial substrates
Mary Coughter

69 - Heavy metals increase antibiotic resistance gene maintenance in the environment
Emilie Dehon

70 - Characterization o Markus Gaenzle f Wastewater Treatment-Adapted Klebsiella pneumoniae: Implications for Environmental AMR and Pathogenicity
Markus Gaenzle

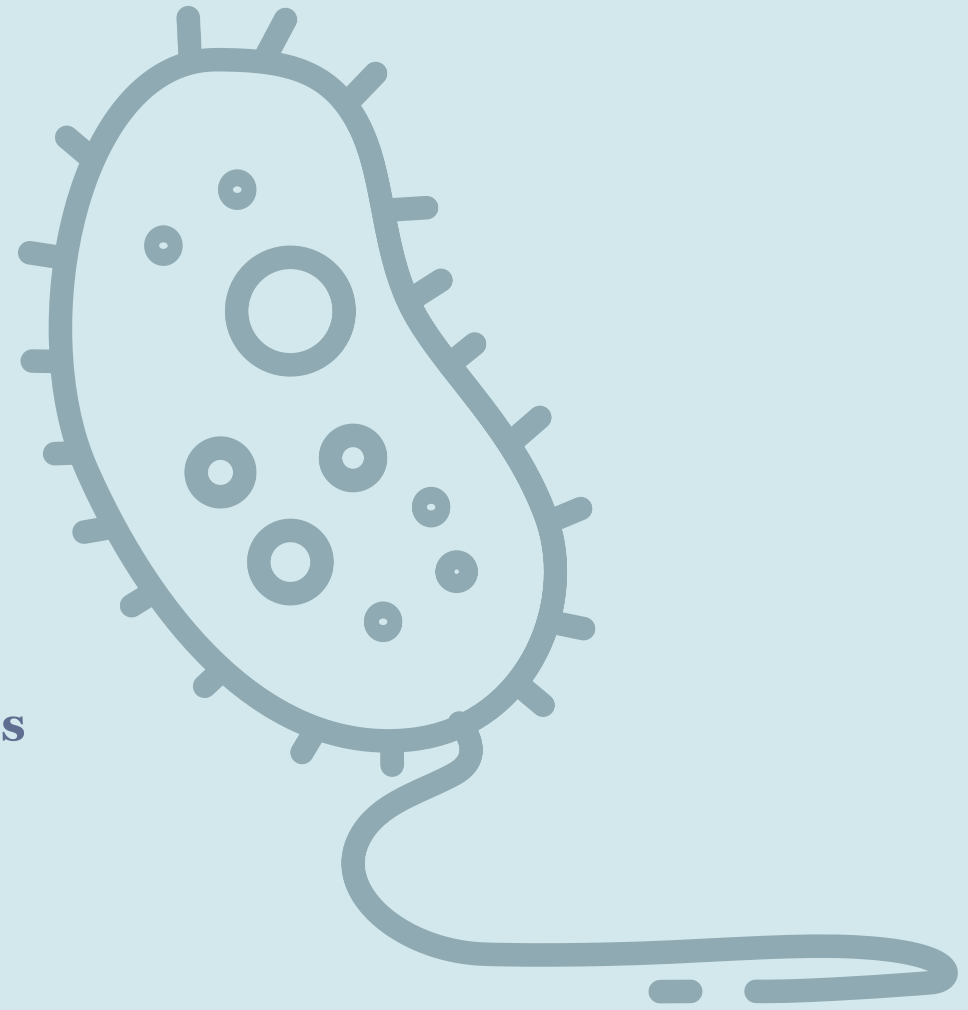
71 - Analysis of antimicrobial resistance genes and plasmid transmissibility in Aeromonas spp. carrying blaIMP-1 isolated from hospital wastewater
Mako Koyama

72 - Assessment of antibiotic resistance across the agro-ecosystem
Alexandra Langlois

73 - Longitudinal ARGs Profiles in Wastewater Treatment Plant Influent Revealed by Metagenomic Analysis
Shuxian LI

74 - Prophage induction by non-antibiotic compounds promotes transformation of released antibiotic resistance genes from cell lysis
JianhuaGuo

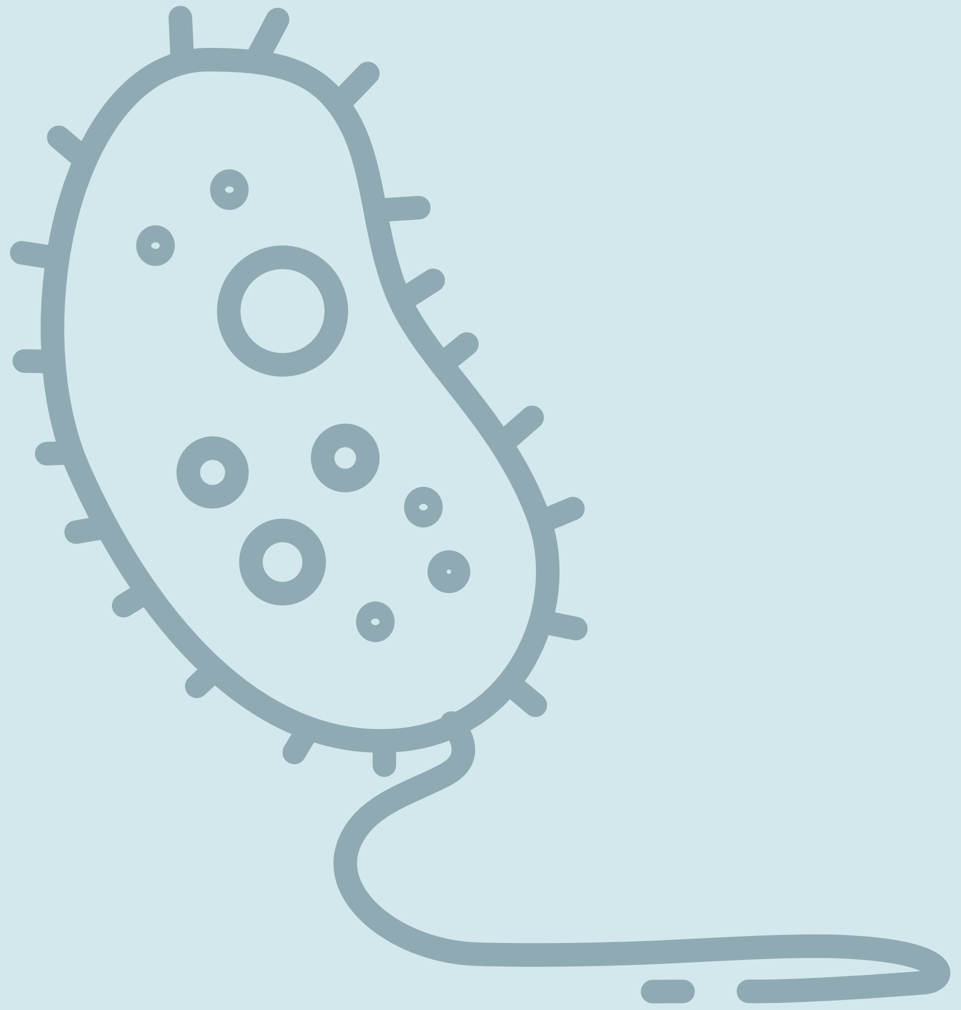
75 - The role of synanthropic birds as sources and potential transmission agents of AMR in Uganda
Charles Masembe



Back to the Program

Poster Session B

77 - Microbiome and Resistome within a First Nation Community and its Surrounding Aquatic Ecosystem in Manitoba, Canada
Md Moniruzzaman
78 - Detection of plasmid-borne copper resistance genes in clinical isolate of Legionella pneumophila using comparative genomics
Maria Najeeb
79 - Antibiotic-resistance genes in bioerosols from wastewater treatment plants: emission and control by air biofiltration
Arthur Ouradou
80 - New epicPCR based system allows tracking of horizontal gene transfer of multiple resistance genes in a microbial community simultaneously
Veera Partanen
81 - Comparative genomics of Escherichia coli ST131 of human, animal and environmental origin from the Czech Republic
Michaela Ruzickova
82 - Antibiotic resistance is maintained in anthropogenically-polluted aquatic ecosystems in the absence of further selective pressure
Concepcion Sanchez-Cid
83 - Do differences in health vulnerability indices between two sewersheds basin impact antimicrobial resistance in sewage of a major Brazil's capital?
Juliana Calábria de Araújo
84 - Wastewater borne pollutants affected the soil resistome and mobilome but not the overall microbial community composition
Kornelia Smalla
85 - Whole-genome analysis of ESBL-producing Escherichia coli and carbapenemase-producing Enterobacteriaceae in sewage collected at manholes
Yu Tang
86 - Genomic Analysis to Determine the Fate of Antimicrobial Resistant Escherichia coli in Soil Amended with Litter from Antibiotic-Fed Broiler Chickens
Paul Waliaula
91 - An Asian Perspective on Antibiotics Contamination in Farm Soil Influencing Antibiotic Resistance Circulation in the Environment
Maria Pythias Espino
92 - Effect of ozonation of municipal wastewater on the reduction of antimicrobial resistance genes (ARG)
Émilie Bédard
93 - The ARISE project: Pioneering wastewater-based AMR surveillance in alignment with upcoming EU regulatory changes
Claudia Kolm
94 - Prevalence and Antimicrobial Resistance Profile of Bacterial Isolates from the Environment of Higher Education Institutions in Maputo, Mozambique
Cristiano Macuamule
95 - THE IMPACT OF ANTIBIOTIC GROWTH PROMOTERS (AGPS) ON BROILER CHICKENS' HEALTH AND ENVIRONMENT IN KIBAHA TOWN COUNCIL – TANZANIA
Damas Theobald Msaki
96 - Reduced antibiotic resistome risks in pig manure after new FDA rule restricting antibiotic use
Xun Qian
97 - Collection of sales and use data of veterinary antimicrobial products in Germany and the European Union
Svenja Sander
98 - Improving the DARTE-QM Monitoring Platform for a One-Health Surveillance Approach
Daniel Gyamfi Amoako
100 - Uncovering the contribution of viruses to antibiotic resistance spreading in paddy soils with manure application
Xin-Li An



Back to the Program

Poster Session B

101 - Strain-level diversity in sulfonamide biodegradation: adaptation of Paenarthrobacter to sulfonamides
Yue Huang

103 - Experiences from wastewater treatment trials using ozone and UV-irradiation to mitigate AMR dissemination from AMR hotspots.
Carsten Ulrich Schwermer

104 - Spatial differences in the human gut microbiota selects distinct antimicrobial resistance genes in a simulated in vitro reactor
Fathima Afsal

105 - First characterization of the prevalence and antimicrobial resistance profile of Salmonella in Quebec sheep flocks
Schlasiva Cenatus

106 - Effect of Advanced-Treated Biosolids Application on AMR in Soil
Clayton Markham

107 - Subinhibitory biocide exposure is more likely to reduce than stimulate conjugal transfer of resistance plasmids
Zhiming He

108 - Elucidating the Transport and Fate of Antibiotic Resistance Genes from Fresh and Treated Cattle Manure in Freshwater Streams
Andrei Badilla-Aguilar

111 - Quantitative microbial risk assessment for waterborne pathogens in a wastewater treatment plant and its receiving surface water body
Sabiha Essack

113 - Impact of an obesogenic diet on the transmission of antibiotic resistance genes from bioaerosols to gut microbiota in mice
ILARIA SCAPINO

114 - Correction effect of Gram positive and Gram negative in extraction and sequencing process based on whole cell spike-in method
Xiang Hui Shi

115 - Exploring python scripts in Geographic Information Systems (GIS) to reveal distribution patterns of antimicrobial resistance in the environment
Patrick Spets

116 - The fate of high-risk antibiotic resistance genes in secondary and tertiary municipal wastewater treatment plants
Guoqing Zhang

117 - Microbial risks triggered through oral administration of antibiotics in fish aquaculture far persist the legally regulated antibiotic withdrawal time
Bing Li

118 - Seeds act as vectors for antibiotic resistance gene dissemination in a soil-plant continuum
Xin-Yuan Li

120 - Antimicrobial Resistance Profiling of Enteric Bacterial Pathogens in Sheep and Goats at a Livestock Show
Ivan Odur

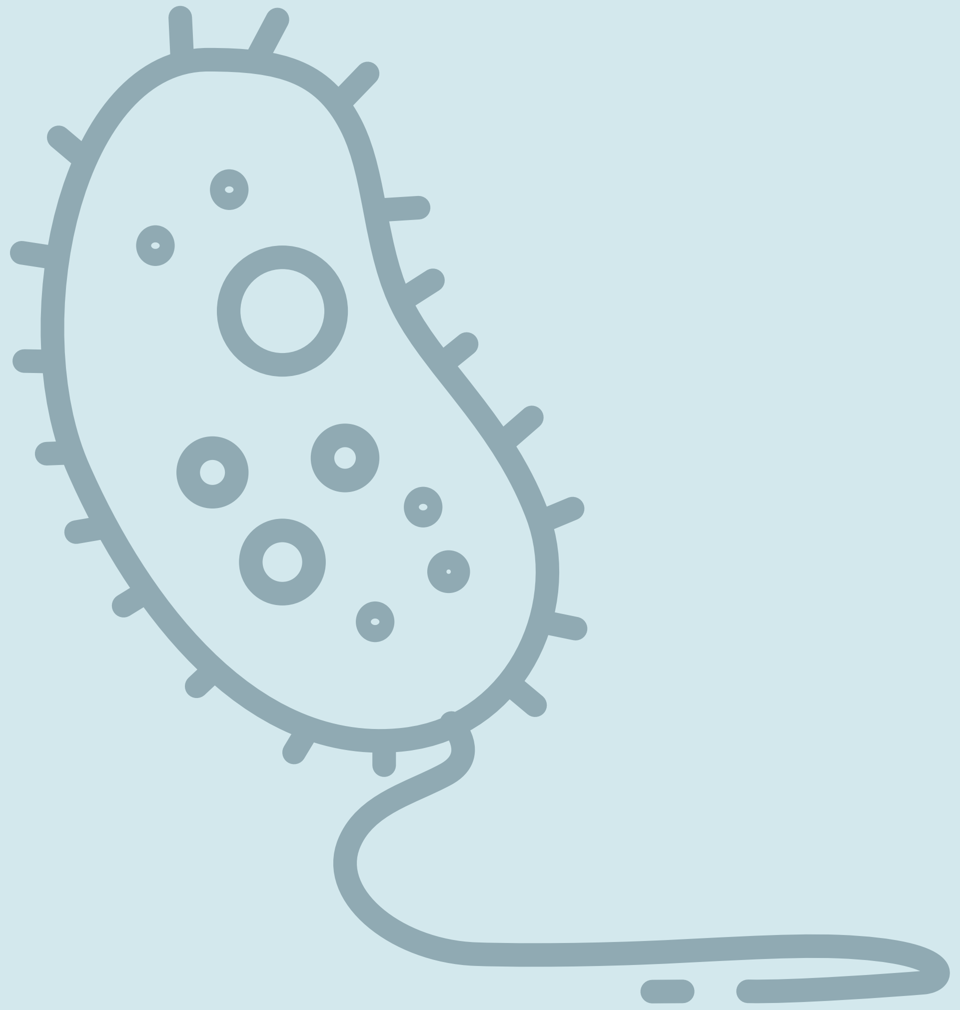
122 - What risk does crop irrigation with reclaimed municipal wastewater represent with respect to the transmission of antimicrobial resistance to humans?
Ed Topp

123 - Unravelling the role of elevated soil temperature and the application of biocides on the spread of antibiotic resistance in organically amended soils
Fernando Ruiz Torrubia

124 - Occurrence and Spread of PPCPs and Antibiotic Resistance in Urban Waters of Sri Lanka
GG Tushara Chaminda

125 - Fate of mobilized colistin resistance genes with increasing concentration of last resort antibiotic in anaerobic membrane bioreactors
Harmita Golwala

131 - Surveillance on dynamic patterns of antibiotic resistant bacteria and multi-drug resistance in Sagami River, Japan
Kazunari Sei



Back to the Program

Poster Session B

132 - Determination of resistance cut-off concentrations for the biocides didecyldimethylammonium chloride (DDAC) and methylisothiazolinone (MIT)
Anne-Christine Schulz

134 - The effect of propidium monoazide (PMA) pre-treatment on qPCR-based pathogen survival monitoring of ozone-treated wastewater effluent
Elfy Ly

135 - Profiling of Resistant Genes in Intermittent Water Distribution Systems and Household Water Storage
Gabriel Mesole

136 - Assessing the impact of anthropogenic pollution on microbial diversity and AMR in recreational waters using metagenomic sequencing
Dearbháile Morris

137 - Paper microfluidic device for rapid detection of antimicrobial resistance in water
Zhugen Yang

140 - Association between particulate matter (PM)2·5 air pollution and clinical antibiotic resistance: a global analysis
Zhenchao Zhou

143 - Understanding Antibiotic Purchasing Practices in Community Pharmacies in Bangladesh: A Potential Driver of Emerging Antimicrobial Resistance.
Abdullah Al Masud

144 - Confronting AMR: A Comprehensive Approach through Public Awareness and Scientific Engagement
Arianna Giurleo

145 - Wastewater genomic surveillance of sexually transmitted infections and their antibiotic resistance in Los Angeles County
Michael Saldana

146 - Opinions of stakeholders on the government, regulator and water industry response to the environmental challenges on the UK AMR National Action Plan
Isobel Stanton

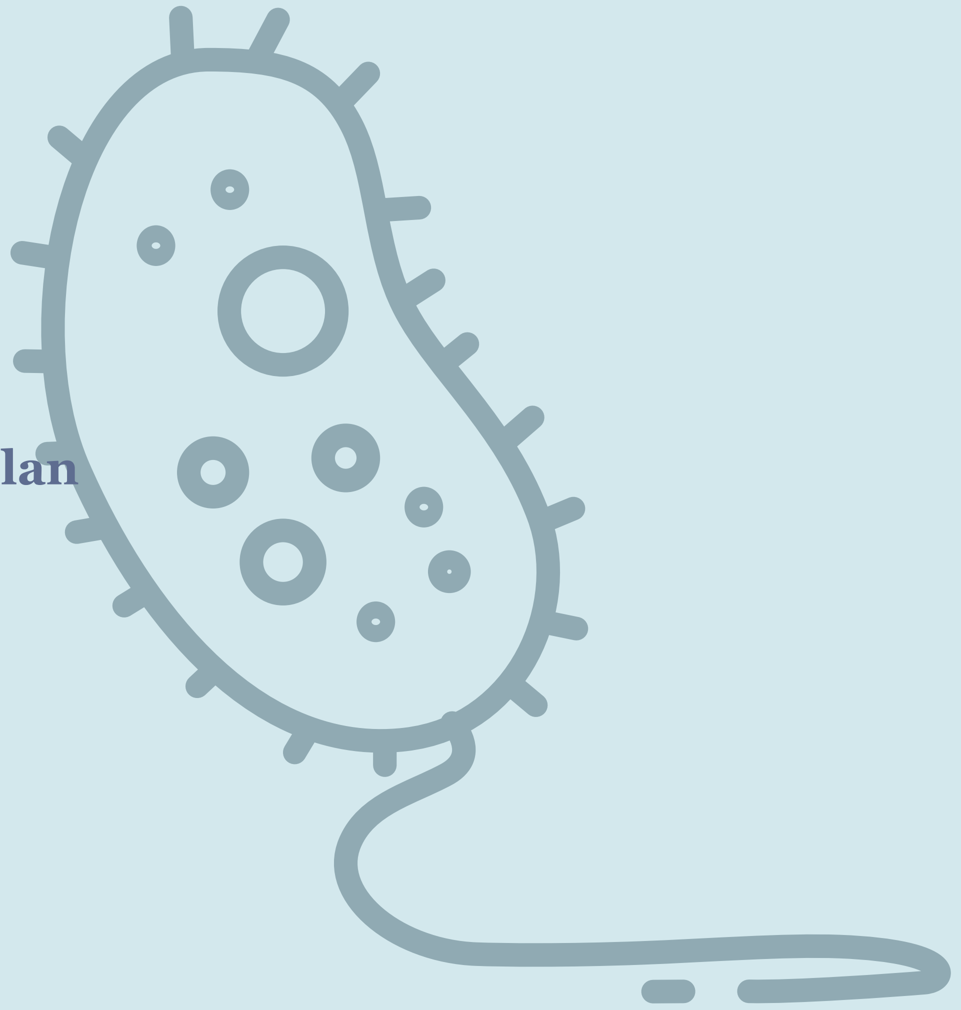
147 - Ultra-Accurate Classification and Discovery of Microbial Protein Functions Using FunGeneTyper: An Extensible Deep Learning-Based Framework
Guoqing Zhang

148 - Genomic analysis of ESBL-producing bacteria recovered from frozen, ready-to-eat, imported stone fruit
Catherine Carrillo

149 - Mining “Big Text” on Antimicrobial Resistance with Natural Language Processing
Shule Li

150 - Factors influencing horizontal dissemination of antibiotic resistance genes
David Lund

151 - Resistance Gene Identifier (RGI) - Prediction of antimicrobial resistance genes in metagenomic sequencing data
Amogelang Raphenya



Back to the Program

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 323

Poster Board number: 1

Impact of wastewater on the invasion of antibiotic resistant bacteria in river biofilms

Background/Aim: Antimicrobial resistance (AMR) ranks among the top 10 global health issues. Microorganisms, particularly bacteria, propagate resistance through molecular mechanisms, contributing to widespread AMR. Factors such as inadequate wastewater treatment and agricultural practices exacerbate this issue. This study was implemented to investigate the influence of filtered wastewater as a stressor on the invasion of antibiotic resistant bacteria (ARB) in river biofilms.

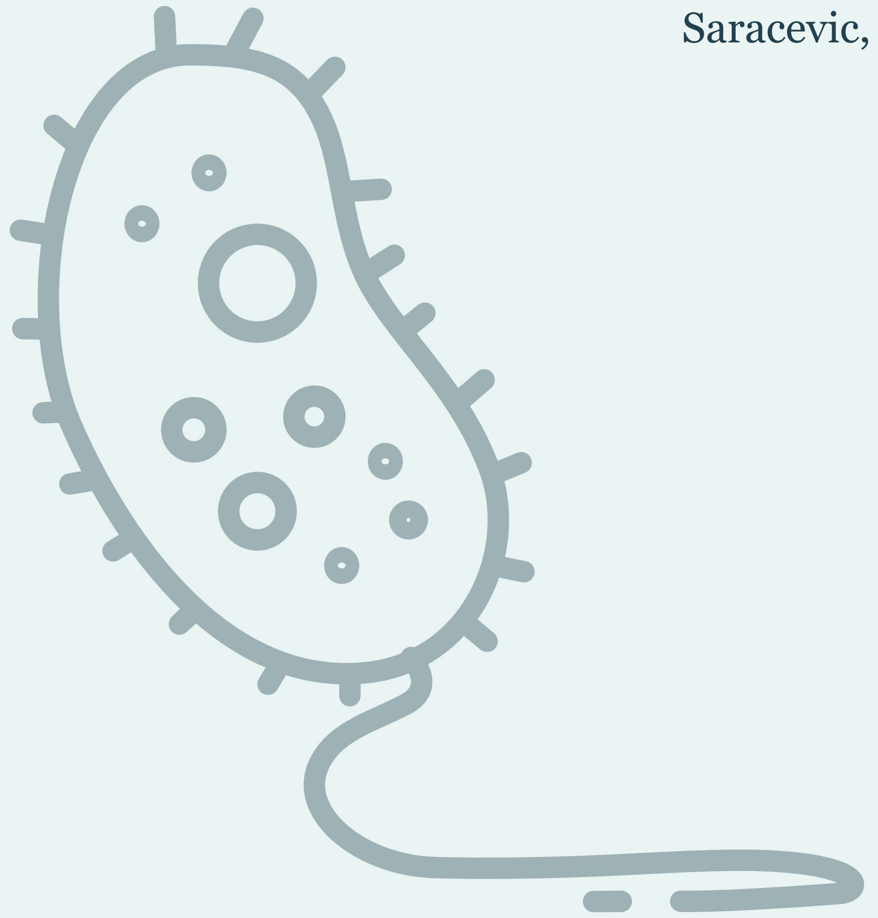
Procedure/Method: Biofilms from a natural aquatic habitat were exposed to river water with 20% filtered (1.0 µm glass fibre filter, 0.45 µm cellulose mixed ester filter) wastewater (influent and effluent) infused with a defined biological invader (E. coli MG1655 tagged on its chromosome and plasmid) for 14 days in a closed lab flume system. With subsequent analysis using qPCR, both the invader and its plasmid were quantified in the biofilm DNA over time to determine how the invasion of ARB and the spread of AMR are influenced by wastewater as a stressor. For a deeper understanding, the wastewater was also analyzed for a range of nutrients, heavy metals and pharmaceuticals.

Findings/Results: We found that the biological invader decreased much slower in one of the two replicates receiving 20% wastewater treatment plant (WWTP) influent as a stressor, with very stable concentrations between day 1 and 2. Such effects could not be determined under the influence of 20% effluent, which showed a similar picture as the control flume without wastewater. Overall, no plasmid transfer could be detected. These results indicate that WWTP influent and the resulting chemical stress imposed to the microbial community can promote the invasion of ARB in river biofilms. In consequence, this is hypothesized to also increase the probability of AMR transmission in the aquatic environments.

Implications/Applications: This experiment thus emphasizes the importance of wastewater treatment as a barrier towards the environmental spread and persistence of AMR.

Author: Irina Dielacher, TU Wien, Austria

Co-Authors: Holzwarth, Helene; Kluemper, Uli; Bellanger, Xavier; Merlin, Christophe; Saracevic, Ernis; Woegerbauer, Markus; Kreuzinger, Norbert; Krampe, Jörg; Vierheilig, Julia



Session A

Abstract 317

Poster Board number: 3

Flash Poster Presentation: S5 - Monday, May 27 - 13:45 - 15:30

Impact of temperature, feedstock, and seasonality on antibiotic resistance genes in hyper-mesophilic anaerobic digestion of agricultural biomass

Background: The production of biogas and biofertiliser from agricultural biomass using anaerobic digestion (AD) is a sustainable practice, however, there are lingering concerns about the propagation of antimicrobial resistance (AMR) in agroecosystems. Although the AD process can reduce antibiotic resistance genes (ARGs), attenuation efficiency varies depending on operating parameters. To inform best practice, it is important to monitor ARGs in full-scale AD. Our preliminary AMR survey examined a unique niche of hyper-mesophilic (40-50°C) AD in the UK. Further, we examined the impact of season-induced temperature/feedstock variation in the hyper-mesophilic range to provide deeper insights into the influence of environmental/operational factors.

Methodology: Primary/secondary digester samples were obtained in the winter/early spring and in the summer from eleven commercial biogas plants operating between 40-50°C. Some digesters were fed exclusively crops while the others were fed a blend of crops, livestock manure, slurry cake, and brewery waste. Genomic DNA was extracted, and shotgun Illumina sequencing was performed.

Findings: In total, 557 ARG subtypes belonging to 26 ARG types were detected across samples. The most abundant types were macrolide-lincosamide-streptogramin, aminoglycoside, tetracycline, polymyxin, and sulfonamide. The ugd gene which confers resistance to polymyxin, a last resort antibiotic, was the most abundant ARG in most sites. Variation in resistome profile was largely associated with feedstock composition and temperature ($p<0.05$, adonis2). A higher proportion of manure in feedstock led to higher ARG abundance. The site that incorporates the highest poultry manure (14-21%), even with pasteurisation, had 4-12 times higher ARG abundance than crop-based sites. Season-induced temperature/feedstock variation resulted in less ARGs in winter than summer for sites incorporating manure, but little impact for crop-based sites.

Implication: The presence of high-risk, clinically problematic ARGs in the digestate demonstrates the need to enhance the in-process and post-digestion attenuation of ARGs by manipulating operational conditions with consideration of seasonality.

Author: Mac-Anthony Nnorom, University of Surrey, United Kingdom

Co-Authors: Short, Michael; Guo, Bing

Back to the Program

Session A

Abstract 271

Poster Board number: 4

Flash Poster Presentation: S3 - Monday, May 27 - 11:00 - 12:30

Large-scale screening of antimicrobial resistance genes along 2300 km of the Danube River

Background: Large rivers are highly impacted by wastewater while also being vital lifelines serving various human needs. A comprehensive understanding of the occurrence, spread and key drivers of antimicrobial resistance (AMR) along whole river courses is largely lacking. Study Design and Methods: We applied a holistic approach to identify spatiotemporal patterns and hotspots of antibiotic resistance genes (ARGs) along 2311 km of the Danube River, Europe, combining a longitudinal and temporal monitoring campaign. The integration of advanced faecal pollution diagnostics (standard faecal indication, microbial source tracking, specific wastewater metric), environmental and chemical key parameters allowed linking ARG concentrations to the major pollution sources and explaining the observed patterns.

Results: Eight ARGs, including genes conferring resistance to five different antibiotic classes of clinical relevance, and one mobile genetic element were determined. All AMR targets could be detected and quantified in Danube River water, with intI1 and sul1 being ubiquitously abundant, qnrS, tetM, blaTEM with intermediate abundance and blaOXA-48, blaCTX-M-1, blaCTX-M-9 and blaKPC genes with rare occurrence. Human faecal pollution from municipal wastewater discharges was the dominant factor shaping ARG patterns along the Danube River at base-flow conditions. ARGs were related to animal contamination mainly during periods with elevated discharge. Other significant relationships of specific ARGs were observed with discharge, certain metals and pesticides. In contrast, intI1 was not associated with wastewater but was already established in the river water microbiome. During temporal monitoring, an extraordinary hotspot was identified emphasizing the variability within natural waters and the necessity to go beyond snap-shot studies.

Implications: This study provides the first comprehensive baseline concentrations of ARGs in the Danube River and lays the foundation for monitoring future trends and evaluating potential mitigation measures. The applied holistic approach proved to be a valuable methodological contribution towards a better understanding of the environmental occurrence of AMR.

Author: Alexander Kirschner, Medical University of Vienna, Austria

Co-Authors: Schachner-Groehs, Iris; Koller, Michael; Leopold, Melanie; Kolm, Claudia; Linke, Rita; Jakwerth, Stefan; Kolarevic, Stoimir; Kracun-Kolarevic, Margareta; Kandler, Wolfgang; Sulyok, Michael; Vierheilig, Julia; Toumi, Marwene; Farkas, Rozsa; Toth, Erika; Kittinger, Clemens; Zarfel, Gernot; Farnleitner, Andreas

Session A

Abstract 121

Poster Board number: 5

Flash Poster Presentation: S5 - Monday, May 27 - 13:45 - 15:30

Antibiotic resistome of final effluents from wastewater treatment plants across China

Background and Aim: Antibiotic-resistant pathogens pose a serious threat to global public health. Discharged effluents from wastewater treatment plants (WWTPs) represent one of the most important sources of environmental AMR. The knowledge and evidence base through surveillance and research is urgently needed to achieve objective 2 of the WHO global action plan on antimicrobial resistance.

Method: Here, by combining metagenomic assembly and binning with culture-based sequencing, the mobility potential, hosts, and potential risk of antibiotic resistance genes (ARGs) in final effluent water from 22 cities (30 WWTPs) across China were characterized.

Findings: We revealed that 85.7% of ARGs co-occurred with mobile genetic elements (MGEs), and ARGs were more prevalent and abundant in plasmids than in chromosomes, suggesting the high mobility potential of resistome in effluent water. Analysis of assembly contigs and metagenome-assembled genomes (MAGs) together revealed that the main host genera were Acinetobacter, Enterobacter, and Pseudomonas. Cultured-based analyses of antibiotic-resistant bacteria (ARB) suggested that with the discharge of effluents pathogenic ARBs were unintentionally released into the environment. We found regional patterns in resistome, which was delineated by a boundary (0-degree isotherm line) that separates regions of southern China from northern China. The health risk of resistome, evaluated by diversity, abundance, mobility, and the potential pathogen hosts, both respectively and integrally, is higher in northern than in southern China.

Implications: This study provides novel insights into the discharge of AMR from WWTPs and its potential risks. It fills the knowledge gap to guide future approaches to mitigating the transmission and contamination of ARGs and antibiotic-resistant bacteria (ARB) in the receiving environments.

Author: Li-Juan Li, Institute of Urban Environment, Chinese Academy of Sciences, China

Co-Authors: Xiao, Yong; Zhao, Bi-Yi; Chen, Geng; Chen, Wei; Yang, Xiao-Ru; An, Xin-Li; Su, Jian-Qiang; Zhu, Yong-Guan

Back to the Program

Session A

Abstract 64

Poster Board number: 6

Flash Poster Presentation: S5 - Monday, May 27 - 13:45 - 15:30

Antimicrobial Resistance in the Adyar River, India, and its Control by Electro-Oxidation

Background and Aim: Antibiotic resistance (AMR) is prevalent in the Adyar River in Chennai, India. This study aimed to quantify ARBs and ARGs to identify sources and hotspots of AMR and seasonal effects. The potential of electro-oxidation to control AMR spread was also studied.

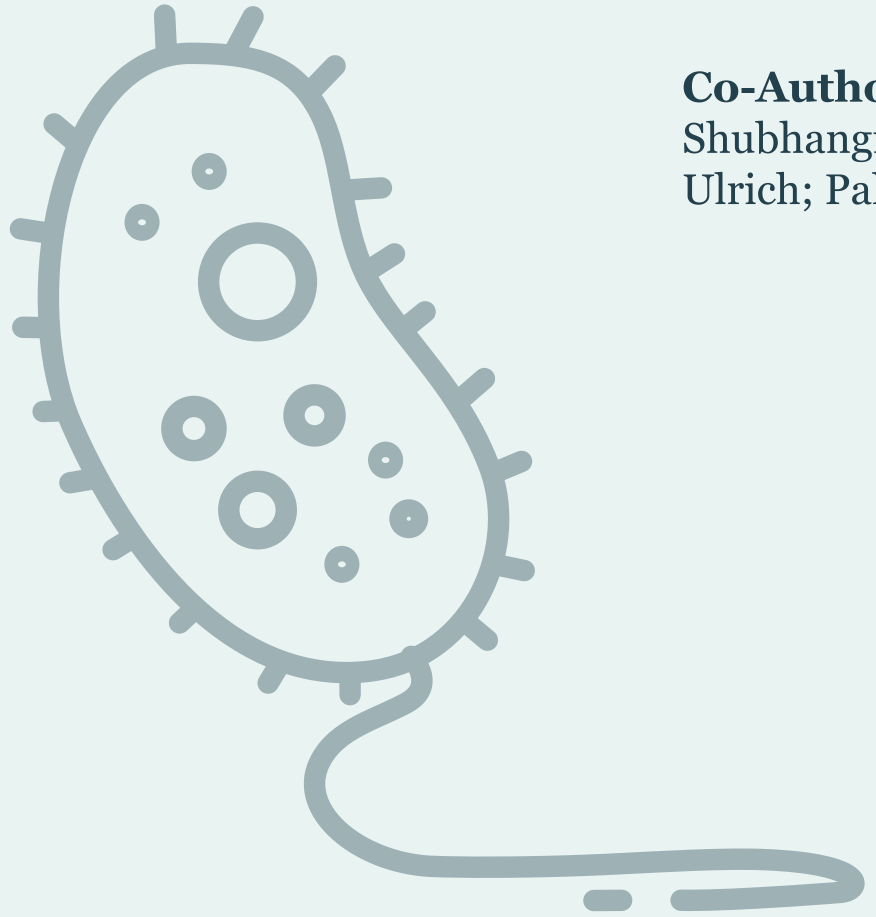
Procedure/Method: Water and sediment were sampled along an 18-km stretch of the Adyar river from upstream to: inside Chennai city in the dry and wet season. Samples were analysed for water quality parameters and ARGs by qPCR and Resistomap HT-qPCR. Resistant and sensitive heterotrophic bacteria and E. coli were counted on synthetic-sewage media or TBX plates with-or-without antibiotics. Resistance patterns in E. coli isolates were also studied. AMR reduction was investigated in 50 L batch-reactors using electro-oxidation and treating secondary-effluent from IITM STP.

Findings/Results: Water quality in the river varied between seasons, which influenced the prevalence of AMR. The seasonal trends of antibiotic-resistant E. coli and heterotrophs differed. Counts on antibiotic-media were higher downstream especially during the dry season. ESBL-carrying E. coli (1.97 Log10CFU/ml) were found only in the downstream wet season samples, probably due to increased pollution downstream. Upstream E. coli isolates had high drug-resistance-indices (MAR) of 0.8 in the dry season (n=13) and 0.62 for both seasons (n=55). qPCR identified 7 representative ARGs, conferring broad-spectrum antibiotic-resistances. ARGs with higher wet season abundance included (all in log10 gene copies/mL): intl1 7.2, sul2 6.35, bla_aph3' 6.64, ermF 5.67, and tetW 4.96, downstream. The reactor studies demonstrated a reduction in ARGs (-1.6 Log10) and CFUs (-2.08 Log10) in 15 minutes. ARGs, ARBs and DNA were undetectable after 20 minutes.

Implications/Applications: The Adyar River is polluted by untreated and treated sewage and harbours diverse ARBs and ARGs. Intl1 genes could enhance AMR spread. We show the potential of electro-oxidation to reduce AMR and protect public health.

Author: THARA M V, Indian Institute of Technology Madras, India

Co-Authors: Vaidyanathan, Rama; Nambi, Indumathi M; Viswanathan, Arathy; Sanjeev, Shubhangi; Rathinavelu, Sasikaladevi; Jothi, Anantha; M, Balaji; J C, Chaitanya; Kreft, Jan-Ulrich; Pallarés Vega, Rebeca; Graham, David; Thatikonda, Shashidhar; Anurag, Subham



Session A

Abstract 44

Poster Board number: 7

Flash Poster Presentation: S3 - Monday, May 27 - 11:00 - 12:30

Linking Antimicrobial Resistance Dissemination to Human Fecal Pollution: A Study on CrAssphage and ARGs in Hospital Wastewater and Environment

Background and Aim: Antimicrobial resistance (AMR) continues to be a widespread global concern, and hospital wastewater plays a significant role as a significant source for its spread within aquatic ecosystems. The objective of this student were to determine the prevalence of antibiotic resistance genes (ARGs) and their correlation with crAssphage, a marker for tracking human-specific faecal sources, in a hospital wastewater treatment plant (WWTP) including upstream and downstream of a receiving canal. Procedure/Method: Water samples were analysed using high throughput quantitative polymerase chain reaction (HT-qPCR) to determine relative abundance of 94 ARGs, crAssphage and 16s rRNA genes, as well as quantitative polymerase chain reaction (qPCR) to determine concentrations of selected ARGs and crAssphage.

Findings/Results: The findings revealed that the blaGES gene was the most relatively abundant in untreated wastewater, although it was found at the lowest relative abundance in the upstream canal, while qacEΔ1, merA, and mobile genetic elements (IS6100, tnpA, and IS26) genes were observed to be relatively prevalent across the treatment processes. Apart from blaVIM, no significant differences were observed in the absolute abundance of most ARGs between treated effluent and environmental samples, implying a widespread distribution of these ARGs in the environment. A strong positive correlation between the absolute concentrations of ARGs, crAssphage, and observed water quality parameters was established. Moreover, the existence of crAssphage facilitated the tracking of human fecal contamination, thus contributing to the environmental dissemination of ARGs.

Implications/Applications: These findings emphasize the importance of monitoring antimicrobial resistance in both hospital wastewater and the adjacent receiving waters, as well as the crucial role of robust biomarkers such as crAssphage in advancing our understanding of the dissemination of ARGs.

Author: Prasert Makkaew, Walailak University, Thailand

Co-Authors: Siri, Yadpiroon; Sresung, Montakarn; Paisantham, Phongsawat; Mongkolsuk, Skorn; Sirikanchana, Kwanrawee; Honda, Ryo; Precha, Nopadol

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 173

Poster Board number: 8

Livestock farm air is a critical pathway in disseminating high-risk antibiotic resistance genes

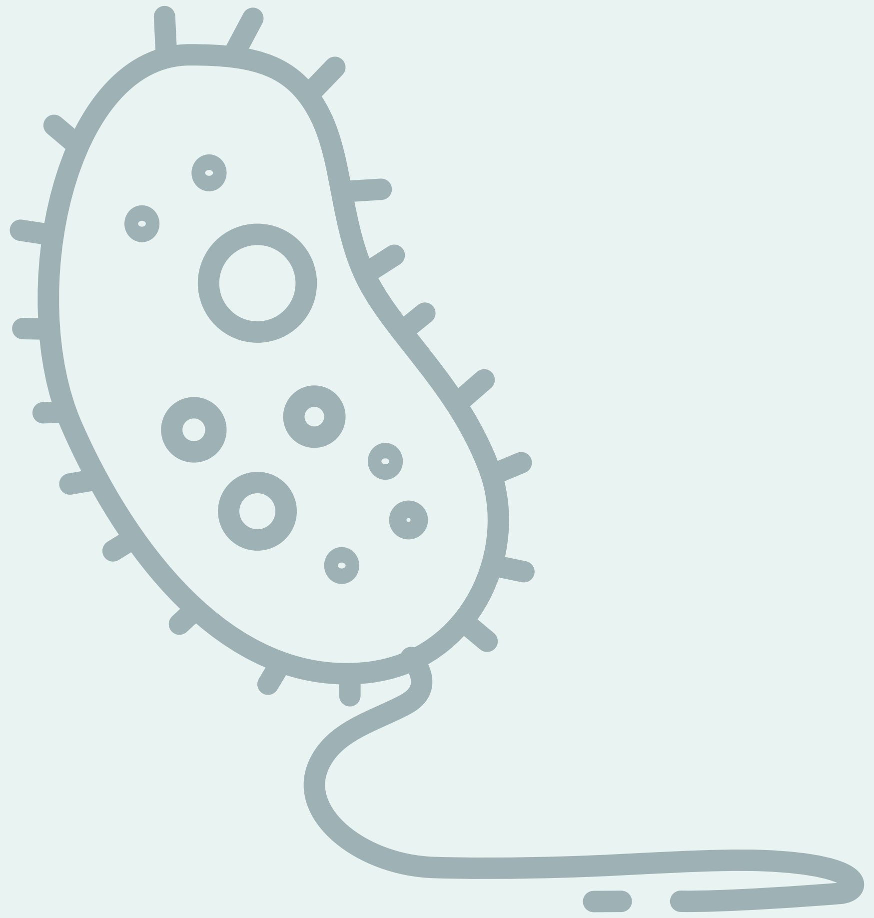
Background and Aim: The majority of global antibiotic consumption is by livestock industry, and livestock environment has become a hotspot of antibiotic resistance genes (ARGs). Airborne contaminants can easily spread at local and regional scales and thus pose direct threats to public health. The health risks of ARGs in livestock farm air remain unclear. Procedure/Method: We used metagenomes from local livestock air samples and from public databases to evaluate and compare the dispersal and projected health risks of ARGs in pig and chicken farm air in China and in European countries.

Findings/Results: We found that livestock farm air and its surrounding air were significantly enriched in ARGs. Chicken and pig farm workers inhaled 527 and 5023 times more ARGs daily than urban residents, while near farm residents (500m distance) inhaled 5–10 times more ARGs than urban residents. The diversity, abundance, potential mobility and MetaCompare risk score of airborne ARGs in China farms were significantly higher than those in the same type of European farms, suggesting the long-term and strict antibiotic use policy in animal farming should mitigate this type of antibiotic resistance exposure. Both culture-based and culture-independent methods demonstrated that many ARGs in livestock air were hosted by pathogens and their close relatives, and their high transferability was further confirmed by conjugation experiments.

Implications/Applications: Our results reveal that livestock farm air is a critical pathway in disseminating high-risk ARGs, and highlights the value of mitigating antibiotic resistance by antibiotic stewardship interventions. This is particularly important for those working inside and living near a livestock housing facility.

Author: James Tiedje, Northwest A&F University, China

Co-Authors: Sun, Wei; Wang, Jun; Qian, Xun



Session A

Abstract 257

Poster Board number: 9

Flash Poster Presentation: S4 - Monday, May 27 - 11:00 - 12:30

Carbapenem-resistant Pseudomonas aeruginosa in hospital wastewater and the water chain in Indonesia and the Netherlands

Background and Aim: Carbapenem-resistant Pseudomonas aeruginosa (CRPA) is a resistant pathogen of critical priority according to WHO. There is limited insight into its presence in the environment and the sources thereof. We investigated the presence and genetic relatedness of CRPA in water samples from hospitals and the receiving environment in both Indonesia and the Netherlands.

Methods: After the development of detection methods, CPRA were quantified monthly in academic hospital wastewater, municipal wastewater as well as the receiving rivers over one year in Jakarta and Rotterdam. Patient isolates were included for comparison. CRPA DNA was sequenced using Oxford Nanopore long-read DNA sequencing on GridION and Illumina short-read DNA sequencing on MiSeq. Genomes were assembled in hybrid mode using NanoLite v1.1 and AMR genes were detected with RGI 6.0.2 with the latest version of the CARD database.

Results: The largest concentrations of CRPA were found in hospital wastewater. In both countries, wastewater treatment reduced CRPA concentrations, but CRPA could still be detected in both treated effluent as well as in the receiving river. In Indonesia, the concentration of CRPA downstream of hospital wastewater was higher than upstream. Three metallo-β-lactamases (blaNDM, blaIMP and blaGES), were detected exclusively in Indonesia in both patients and communal water, while blaVIM was detected in all sampled reservoirs, in both countries. However, a large majority of strains that were phenotypically carbapenem-resistant did not harbor sensu stricto carbapenemase genes. While core genome multi-locus typing is still ongoing, clonal hospital/water isolate pairs have been identified.

Implications: This study shows that hospitals act as a source of CRPA entering the aquatic environment, and evaluates the impact of different wastewater management strategies (onsite hospital wastewater treatment in Indonesia and the Netherlands, next to municipal wastewater treatment in the Netherlands) on the spread of CRPA.

Author: Selvi Nafisa Shahab, Department of Medical Microbiology and Infectious Diseases, Erasmus MC University Medical Centre, Rotterdam, The Netherlands; Department of Clinical Microbiology, Faculty of Medicine Universitas Indonesia / Dr. Cipto Mangunkusumo Hospital, Jakarta, Indonesia, Indonesia

Co-Authors: van Veen, Anneloes; Nguyen, Guillaume Quang Henri; Gauthier, Jeff; Kukavica-Ibrulj, Irena; Potvin, Marianne; Kemper, Merel A.; Rijfkogel, Amber; Karuniawati, Anis; Lévesque, Roger C.; Severin, Juliëtte A.; Klaassen, Corné H. W.; Schmitt, Heike

Back to the Program

Session A

Abstract 53

Poster Board number: 10

Dynamics of Extracellular Antimicrobial Resistome in Full-scale Membrane Bioreactor and Conventional Activated Sludge Processes

Background and Aim: Wastewater constitutes a major reservoir of antimicrobial resistance (AMR) in the environment, with extracellular antimicrobial resistance genes (eARGs) being notably abundant. These genes contribute to the proliferation of AMR through horizontal gene transfer (HGT). This study aims to investigate the fate of eARGs and intracellular ARGs (iARGs) in both conventional activated sludge (CAS) and membrane bioreactor (MBR) processes at a full-scale wastewater treatment plant (WWTP).

Method: Seasonal samples from influent wastewater, activated sludge, and treatment effluent were collected at a full-scale WWTP in Japan. Following sample fractionation into intracellular and extracellular components, extracted DNA underwent shotgun metagenomic sequencing. ARG composition was determined by aggregating and normalizing reads from each ARG after blasting with CARD v.3.2.6. Results: The proportion of eARGs to iARGs gradually increased from influent (16-28%) to effluent (40-74%), attributed to a decline in iARG abundance. However, the abundance of eARGs per 16S bacterial population exhibited no significant change throughout both CAS and MBR processes. The composition of eARGs in influent wastewater mirrored iARGs, but a substantial shift occurred from influent to activated sludge. The latter's eARG composition persisted in treatment effluent regardless of process or season. In contrast, iARG composition showed minimal change. Notably, eARG composition in activated sludge and effluent was less diverse and dominated by aadA family ARGs related to aminoglycosides, while the qacL, multidrug ARG on disinfection agents and antiseptics, was also prevalent in spring and summer. MBR demonstrated a higher reduction in both eARGs and iARGs compared to CAS, primarily due to a more substantial reduction in the total bacterial population.

Implication: This study unveils the selective retention of specific eARGs in activated sludge, subsequently discharged via WWTP effluent. Moreover, the MBR system emerges as a promising strategy for minimizing the dissemination of eARGs through WWTP effluent.

Author: Mardalisa -, Division of Environmental Design, Graduate School of Natural Science and Technology, Kanazawa University, Japan

Co-Authors: Wang, Rongxuan; Sabar, Muhammad Adnan; Honda, Ryo; Kamoshida, Hiroya

Session A

Abstract 243

Poster Board number: 11

Sub-minimum inhibitory concentrations of florfenicol and tetracycline enhanced horizontal transfer of AMR genes between Salmonella strains

Sub-minimum inhibitory concentrations of florfenicol and tetracycline enhanced horizontal transfer of antimicrobial resistance genes between Salmonella strains

Background and Aim: Antimicrobial residues are highly prevalent in veterinary and agricultural environments and promote the growth of antimicrobial resistance (AMR) bacteria. However, it is not clear if antimicrobial residues will influence the horizontal transfer of AMR genes and lead to further spread of AMR. Therefore, our research goal is to test whether exposure to sub-MIC doses of antibiotics promotes the horizontal transfer of AMR genes.

Procedure/Method: We evaluated the conjugative transfer of an Inc A/C plasmid that carries multiple AMR genes using Salmonella enterica serovar Heidelberg as a donor and S. Typhimurium as a recipient. The minimum inhibitory concentrations (MICs) of tetracycline and florfenicol for both strains were determined. Conjugation of the donor and recipient was performed with filter mating on Luria-Bertani (LB) agar containing 1/2, 1/4, and 1/10 of the MIC of florfenicol and tetracycline of the recipient.

Findings/Results: The plasmid transfer efficiency was significantly higher on all sub-MICs of tetracycline and the highest sub-MIC of florfenicol than that on plain LB agar. Implications/Applications: Our findings suggest that exposure to antimicrobial residues might enhance the transfer of AMR genes and contribute to the spread of AMR.”

Author: Amer Abdelgany, Canadian Food inspection Agency (CFIA), Canada

Co-Authors: Guan, Jiewen

Session A

Abstract 209

Poster Board number: 13

Flash Poster Presentation: S6 - Monday, May 27 - 13:45 - 15:30

The alteration of microbial biodiversity in the receiving environment promotes its invasion by anthropogenic antibiotic resistance genes

Background and Aim: The invasion of natural communities by anthropogenic antibiotic resistance genes (ARGs) relies on both the persistence of the resistant bacterial invaders and their ability to transfer their ARGs to the receiving communities. Even if the pervasiveness of ARGs in human-impacted environments is evident, and horizontal gene transfer “”easily”” demonstrated in vitro, the invasion dynamics of natural communities by ARGs remain difficult to observe, thus suggesting a strong biotic resistance. The aim of this work was to demonstrate the influence of the biodiversity of natural receiving communities in holding back or favoring their invasion by ARGs.

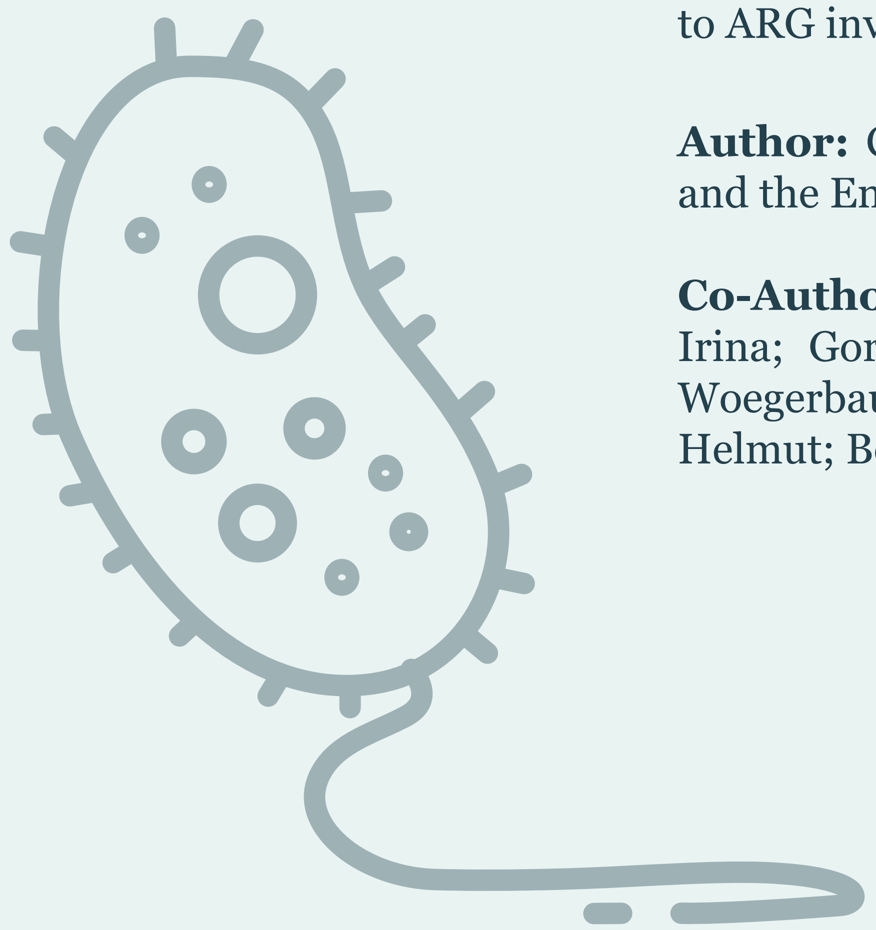
Procedure/Method: This work focuses on epilithic biofilms communities residing in rivers. High-throughput qPCR of 50 ARGs and 16S rRNA gene amplicon sequencing were used to quantify invading ARGs and the biodiversity of biofilm communities along river continuums. The invasion dynamics of natural biofilms collected in these rivers was also monitored in reactors after spiking a defined bacterial invader tagged beforehand on its chromosome and its broad host range conjugative plasmid. Both invader and plasmid were quantified in biofilm DNA by qPCR over time.

Findings/Results: The analysis of river continuums demonstrated that a third of the ARGs tested were absent from the ecosystems, another third was already present everywhere, from pristine-like environments to downstream anthropogenically impacted areas, and the final third resulted from the massive invasion of biofilm communities upon crossing a prime exposure to human activities (hamlet, pastures). Invasion by ARGs appeared higher as the biodiversity of the river biofilm decreases. Transplanting these biofilms in reactors, invasion assays using a model invader indicated that (i) no plasmid transfer could be detected by molecular means, but (ii) the persistence of the invader increased significantly as the biodiversity of the receiving biofilm decreased.

Implications/Applications: Preserving environmental biodiversity may promote barrier effects to ARG invasion.

Author: Christophe Merlin, Laboratory of Chemical Physics and Microbiology for Materials and the Environment - University of Lorraine / CNRS, France

Co-Authors: Catao, Elisa; Klemper, Uli; Gionchetta, Giulia; Bellanger, Xavier; Dielacher, Irina; Goryluk-Salmonowicz, Agata; Szekeres, Edina; Changey, Frédérique; Walsh, Fiona; Woegerbauer, Markus; Coman, Cristian; Popowska, Magdalena; Vierheilig, Julia; Bürgmann, Helmut; Berendonk, Thomas U.



Session A

Abstract 57

Poster Board number: 14

Antibiotic resistance dimension in Belo Horizonte, Brazil, through the lens of hospitals and communities wastewaters

Background and Aim: Hospital wastewater connects clinical and natural environments, reflecting patients with bacterial infections or undergoing antibiotic treatment. Antibiotic-resistant infections have been observed in the general community. The wastewater that reaches Wastewater Treatment Plants (WWTPs) is produced by the population, thus serving as a valuable representation of the community. This study conducted surveillance of antimicrobial resistance (AR) in wastewaters in Belo Horizonte, one of the largest cities in Brazil, which is among the countries that consume the most antibiotics in the world.

Procedure/Method: This study occurred in two sewage basins with varying socioeconomic profiles. Around 82% of the municipality’s sewage is collected and directed to the two WWTPs (A and B) investigated. Hospitals (A) and (B) directly discharge their sewage into the sewage network, which then flows to WWTPs (A) and (B), respectively. Genes conferring resistance to sulfonamides, macrolides, quinolones, extended-spectrum beta-lactams, as well as the class 1 integron gene were identified and quantified in hospital and community wastewater monthly for one year by qPCR.

Findings/Results: Hospital sewage significantly differed from community sewage in terms of all examined AR genetic elements ($p<0.05$). In all four locations, average concentrations followed this order: sul1> Intl1> blaTEM or qnrB> ermB (10^{6}-10^{9} copies.mL□^{1}), surpassing averages reported from other countries. When comparing with previous data from the last three years, concentrations in the municipality’s community sewage increased. A significant difference ($p<0.05$) in genetic concentrations between community and hospital sewage from different basins was evident only for the quinolone resistance gene. Seasonal variations did not considerably impact the abundance of these genetic markers.

Implications/Applications: This study revealed the magnitude of the AR problem in the municipality and the need for better wastewater management, especially from hospitals facilities, to reduce the environmental spread of AR. Environmental surveillance in wastewater plays a key role in providing crucial information to guide policies and actions aimed at mitigating AR.

Author: Ana Paula Assad de Carvalho, Universidade Federal de Minas Gerais, Brazil

Co-Authors: Calábria de Araújo, Juliana; Silva Almada, Mariana; Pereira Queiroz, Ana Luiza; Dutra Leal, Cintia; Cristina Machado, Elayne

Back to the Program

Session A

Abstract 171

Poster Board number: 15

Tracking ARGs through a Potable Water Reuse Treatment Train

Background and Aim: Water reuse is a growing necessity to advance water sustainability, but research is needed to determine which treatment configurations most effectively mitigate antimicrobial resistance concerns. Treatment trains that employ biological treatment are of special interest as a potential reservoir for antibiotic resistance genes (ARGs). Here we utilized shotgun metagenomic sequencing to track the fate of ARGs throughout a potable reuse train employing coagulation, flocculation, sedimentation, ozonation, biologically-active-carbon (BAC) filtration, granular-activated-carbon (GAC) contacting, and UV disinfection. Spatial and temporal shifts in the resistome were related to fluctuations in process performance and changes in operational conditions.

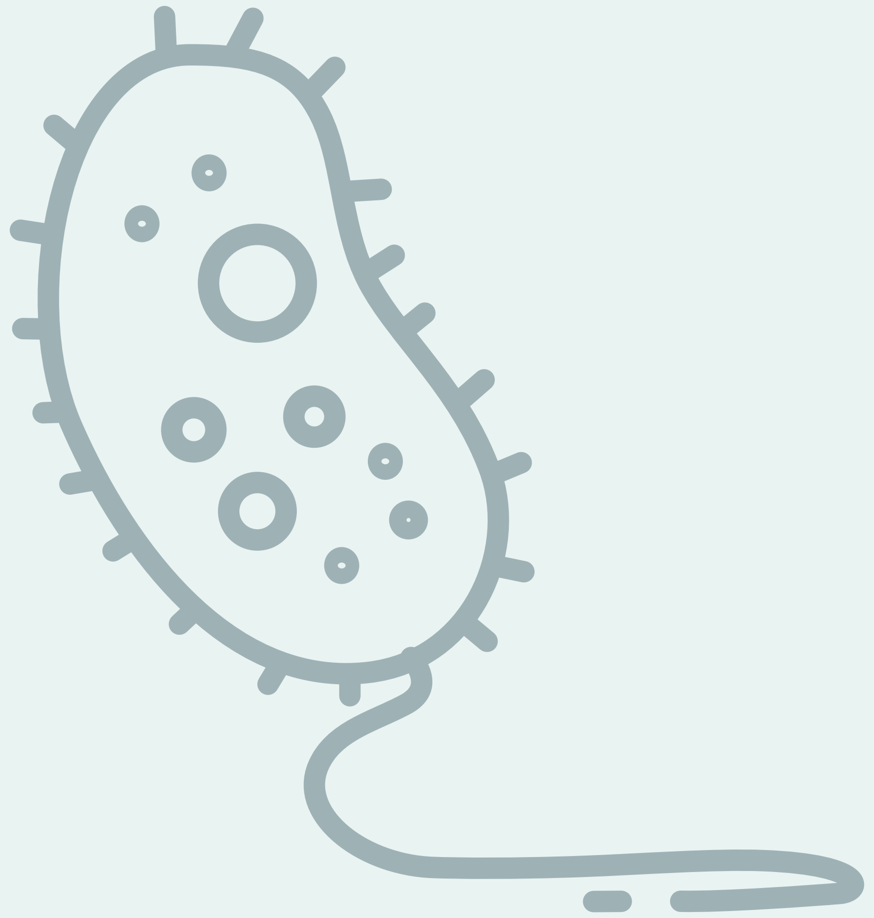
Procedure/Method: Monthly samples were collected from each stage of treatment at a 4.3 GPM pilot plant and extracted DNA was subject to shotgun metagenomic sequencing via Illumina NextSeq500 and the NEB Ultra II library prep. ARGs were annotated using a manually-curated version of the CARD database and compared via ANOSIM, ADONIS, Dunn's T Test, and paired Wilcoxon rank sum test to evaluate the extent to which treatment processes and operational conditions shape the resistome of the treated water.

Findings/Results: Disinfection processes provided effective reduction of volumetric abundances of ARGs (copies/mL), but elevated relative abundances, suggesting that they impose selection pressure for resistant bacteria. The resistomes clustered by stage of treatment (ANOSIM, p-value<0.05, r-stat = 0.67|0.42), moreso than any other tested factor.

Implications/Applications: Overall, the treatment train was found to effectively reduce the loading of total ARGs, but resistant bacteria may differentially survive disinfection processes. The findings can help to ensure that water reuse treatment trains adequately address concerns about antibiotic resistance.

Author: Matthew Blair, Virginia Tech, United States

Co-Authors: Pruden, Amy; Bott, Charles; Vikesland, Peter



Session A

Abstract 307

Poster Board number: 17

Flash Poster Presentation: S3 - Monday, May 27 - 11:00 - 12:30

Key Elements within an Integrated Monitoring Framework of Antimicrobial Resistance for Asian Countries

Background and Aim: There is an urgent need for an integrated antimicrobial resistance (AMR) monitoring framework in low- and middle-income countries (LMICs) in Asia. Recognizing low resource settings and local contexts in Asian LMICs, we aimed to provide suggestions on: what to monitor, how to monitor, and where to monitor AMR in the environment.

Procedure: An expert panel discussion, including representatives from India, Thailand, Indonesia, Sri Lanka, China, and Japan, was held in March 2023 to design an appropriate AMR monitoring framework. This was further refined by the Asia-Pacific Researcher Network on Environmental Dimensions of Antimicrobial Resistance (EnD-AMR-Asia), consisting of 20 experts from 9 countries.

Results: Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli, a key indicator in the WHO Tricycle protocol, is suggested as a primary target for monitoring. This choice is due to its frequent detection in various environmental matrices in Asia and the availability of a comprehensive genomic database for tracking its origins. In terms of methodology, culture-based methods are favored for their cost-effectiveness and widespread feasibility in Asian LMICs. Existing laboratory and human resources are available for these methods. Molecular-based methods are suggested as supplementary, particularly in countries with the necessary facilities, to enhance understanding of AMR sources, diversity, and development. Regarding location, rivers are suggested as strategic sites for nationwide AMR monitoring, covering large areas with contributions from a variety of sources. Untreated wastewater is another priority target in urban and tourist areas. The frequency often involves a trade-off with the number of locations; however, at least annual sampling is recommended.

Implications: There is a critical need for implementing AMR monitoring in water environments across Asian countries. We advocate for a globally applicable environmental monitoring framework for AMR surveillance, aiming to integrate perspectives from various countries and stimulate global discussion on this vital issue.

Author: Ryo Honda, Kanazawa University, Japan

Co-Authors: Kumar, Manish; Wang, Rongxuan; -, Mardalisa; Sabar, Muhammad Adnan; Chaminda, Tushara; Sirikanchana, Kwanrawee; Makkeaw, Prasert; -, Sulfikar; Ju, Feng; Jiang, Guangming; Li, Bing; Chiemchaisri, Chart; Gomi, Ryota; Amarasiri, Mohan; Venter, Henrietta; Nishiyama, Masateru; Watanabe, Toru; Ihara, Masaru; Kasuga, Ikuro; Watanabe, Kozo; Suzuki, Satoru

Back to the Program

Session A

Abstract 73

Poster Board number: 18

Investigation of antimicrobial resistance in sewage and effluent from stabilization lagoons in the Belo Horizonte region, during the COVID-19 pandemic

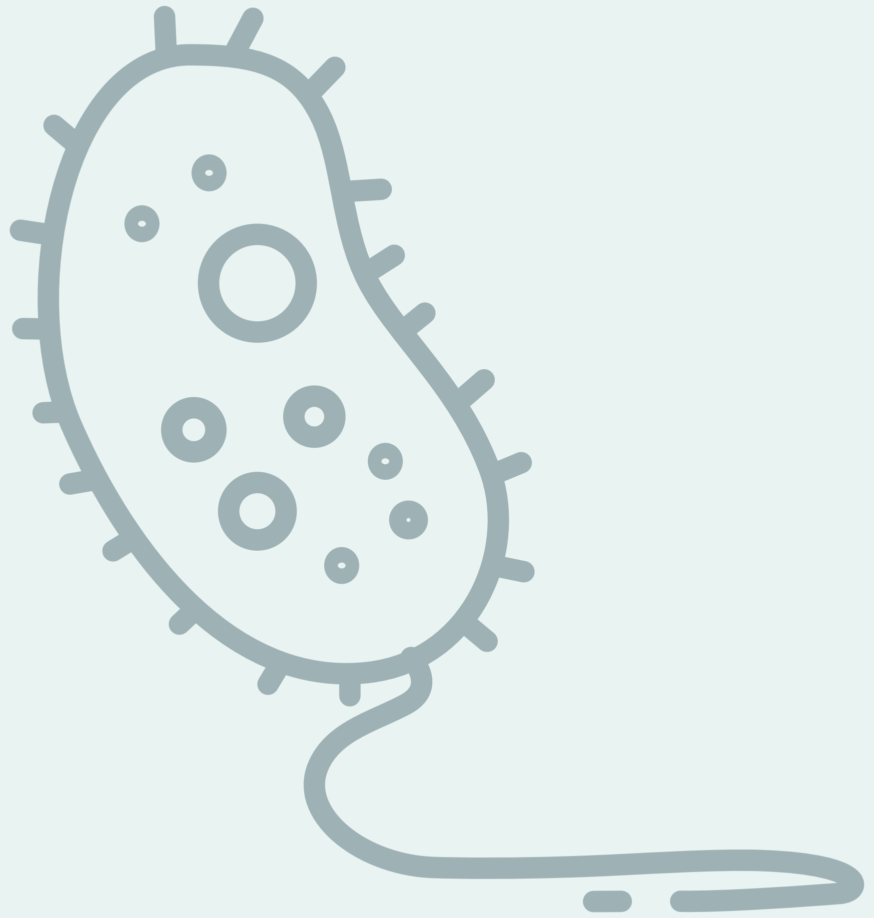
Background and Aim: Wastewater treatment plants (WWTPs) are the main barriers to the release of pollutants, antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (GRAs) into nature, being crucial in protecting human and environmental health1,2

Procedure/Method: This work investigated the occurrence of ARBs and GRAs in raw sewage from a WWTP, in the metropolitan region of Belo Horizonte/MG, during the COVID-19 pandemic, in samples collected weekly (between October/2021 to June/2022), also evaluating the impact of the pandemic on the spread of antimicrobial resistance. The quantification and identification of ARBs cultivable with Amoxicillin, Azithromycin, Cephalexin, Meropenem, Sulfamethoxazole+trimethoprim was carried out, comparing the concentrations of ARBs found in raw sewage, with the concentrations determined in previous work, before the pandemic. Furthermore, the removal efficiencies of total heterotrophic bacteria (THB), ARBs and GRAs were evaluated in the treatment of sanitary sewage in a facultative lagoon system, followed by a maturation lagoon.

Findings/Results: The highest removal efficiency observed was for bacteria resistant to sulfamethoxazole+trimethoprim (99.7%, 2.5 log units); while the lowest efficiency (93.5%, 1.2 logs) was for meropenem-resistant bacteria. The highest removal values found were for the resistance genes blaTEM, ermB and qnrB, corresponding to 3.05 log, 3.08 log and 3.12 log, respectively. The lowest removal values were for sul1 and tetA, corresponding to 0.89 log and 0.72 log, respectively. Implications/Applications: Among the results obtained, it is possible to highlight that the ARBs most frequently identified in raw and treated sewage samples were: Escherichia coli, Citrobacter freundii, Enterobacter asburiae, Enterobacter cloacae, and Pseudomonas otitis. At higher concentrations of ARBs and GRAs compared to other studies3,4, it is possible to conclude that the COVID-19 pandemic had an impact on the concentrations of THBs, ARBs and GRAs studied.

Author: Juliana Calábria de Araújo, Universidade Federal de Minas Gerais, Brazil

Co-Authors: Coelho, Gabriela; Santos, Henrique



Session A

Abstract 59

Poster Board number: 19

Removal of antibiotic resistance genes in full-scale wastewater treatment plants using aerobic and anaerobic systems in Brazil

Background and Aim: Wastewater Treatment Plants (WWTPs) are considered interfaces between society and the environment and one of the most important environmental reservoirs and sources of antibiotic resistance (AR). These units bring together antibiotic-resistant bacteria, antibiotic residues, and other potential promoters of the occurrence of horizontal gene transfer, further facilitated by biological treatment.This facilitation is attributed to the elevated microbial density and nutrient abundance in this setting. This work investigated the removal efficiency of ARGs in two WWTPs that use different biological treatment technologies.

Procedure/Method: Over the course of one year, influent and effluent wastewater samples were collected monthly from two WWTPs located in Belo Horizonte, Brazil. WWTP(A) and WWTP(B) have flow rates of 2,300 and 2,089 $L.s^{-1}$ equivalents populations of 1,401,584 and 1,339,962 inhabitants, respectively. WWTP(A) utilized Conventional Activated Sludge and WWTP(B) employed UASB (Upflow Anaerobic Sludge Blanket) followed by TF (Trickling Filter). The detection and quantification of genes that confer resistance to sulfonamides, macrolides, quinolones, and extended-spectrum b-lactams, as well as the class 1 integron gene were carried out using qPCR.

Findings/Results: WWTP(A) showed better performance (0.32–2.64 log removal), significantly removing ($p<0.05$) all ARGs investigated. In WWTP(B), the removals obtained varied between (-1.87) and 2.08 logs, highlighting the “negative” efficiencies that occurred for most genes in four of the months investigated. In general, the removals of ermB, blaTEM and qnrB were greater than those of Intl1 and sul1 in both WWTPs. ARG concentrations in treated effluents were higher than those reported in the literature from other countries using the same treatment technologies.

Implications/Applications: This study exhibited the differences in the removal behavior of ARGs in WWTPs operating with different technologies. Understanding the specific constraints and capabilities of each treatment technology is critical to advancing and improving these facilities to reduce the spread of AR in the environment.

Author: Ana Paula Assad de Carvalho , Universidade Federal de Minas Gerais, Brazil

Co-Authors: Calábria de Araújo, Juliana; Silva Almada, Mariana; Pereira Queiroz, Ana Luiza; Dutra Leal, Cintia; Cristina Machado, Elayne

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 135

Poster Board number: 20

Development of Copper Resistance in Legionella pneumophila via Experimental Evolution

Background and Aim: Copper-silver ionization, the periodic release of copper and silver ions, is used to eliminate the opportunistic pathogen Legionella pneumophila from engineered water systems. However, facilities utilizing this treatment method have seen the recolonization of the systems by L. pneumophila over time. Further examination showed L. pneumophila populations within these systems were resistant to both silver and copper. Additionally, copper resistant lineages of L. pneumophila have been isolated from biofilms in copper pipes within engineered water systems. These resistant isolates differed from copper-sensitive isolates within the same water system by only 29 SNPs, suggesting that resistance emerged as the result of the accumulation of individual point mutations.

Method: To mimic this process in a controlled environment, an adaptive laboratory evolution model was used to adapt a wild-type strain of L. pneumophila for survival in the presence of increasing concentrations of copper ions. Results: Initially, L. pneumophila populations were exposed to 8 mM $CuCl_2$. Over their evolution, these populations became increasingly resistant to $CuCl_2$, eventually becoming resistant to 16 mM $CuCl_2$. Control lineages that were passaged but not exposed to copper had a significantly lower survival rate in the presence of $CuCl_2$ than the evolved lineages. The genomes of the copper resistant lineages will be sequenced via whole genome sequencing and analyzed with breseq to identify point mutations that confer copper resistance.

Implications: This research demonstrates that the evolution of resistance in L. pneumophila may occur through the accumulation of individual point mutations as the result of repeated exposure to bacterial control methods.

Author: Gillian Cameron, McGill University, Canada

Co-Authors: Faucher, Sebastien

Session A

Abstract 294

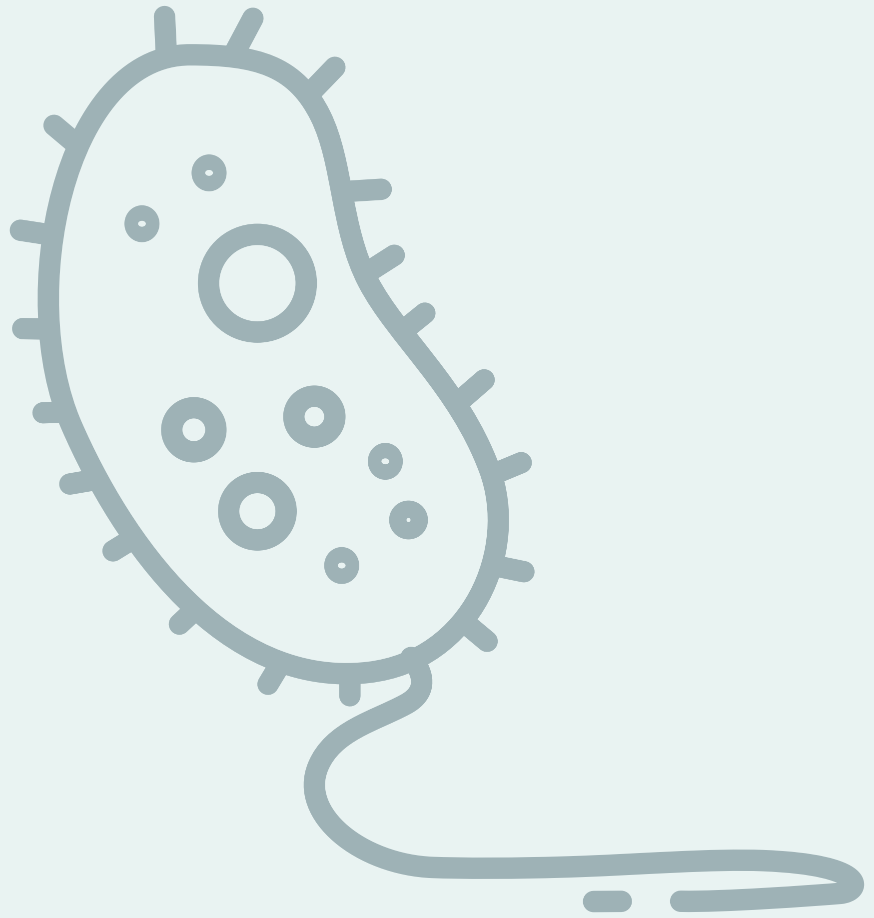
Poster Board number: 21

Loss of microbial diversity and abundance of resistomes during termite mound formation

Termites, as soil-dwelling macrofauna, are among the most abundant organisms globally, particularly in tropical and subtropical regions where they construct extensive nests known as 'mounds.' These mounds significantly alter soil properties and enhance nutrient cycling. Despite the differences between termite mound environments and surrounding bulk soils, little is known about how termite nesting activity influences the distribution and diversity of soil microbial communities, as well as the spread of antibiotic resistance genes (ARGs). This knowledge gap creates uncertainty in predicting ecosystem functions and potential risks associated with termite mounds in a changing environment. Here, we conducted a large-scale (> 1500 km) investigation in northern Australia and found distinct resistomes profiles in termite mounds and bulk soils. Our study provides novel evidence that termite mounds harbor unique microbial assemblages with a lower relative abundance of rare taxa compared to bulk soils. The termite nesting process acts to potentially enrich the dominant soil taxa while filtering out the rare taxa, resulting in a significant decrease in microbial diversity for both bacteria and fungi. Furthermore, our profiling of a wide spectrum of ARGs revealed that the abundance and diversity of ARGs were notably lower in termite mounds compared to bulk soils. Specifically, the proportion of efflux pump ARGs was significantly lower in termite mound resistomes than in bulk soil resistomes. Considering the decrease in microbial diversity, particularly the relative abundance of rare taxa due to the termite nesting process, we infer that rare taxa may have a higher proportion of harboring resistomes compared to abundant taxa. Together, our results highlight the necessity to consider the roles of soil faunal activities in regulating resistomes in terrestrial ecosystems, which would facilitate the development of strategies in fighting against antibiotic resistance in natural ecosystems in the changing environment.

Author: Qinglin Chen, Institute of Urban Environment, Chinese Academy of Sciences, China

Co-Author: Hu, Hangwei



Back to the Program

Session A

Abstract 144

Poster Board number: 22

Flash Poster Presentation: S2 - Monday, May 27 - 8:30 - 10:15

Below the surface: The hidden consequences of eutrophication on river resistome, mobilome and virulome

Background and Aim: Eutrophication, resulting from runoff of nitrate and phosphate from farmlands, and municipal and industrial discharge, is a key factor in the global decline of freshwater and coastal marine ecosystems. The increased frequency of eutrophication events is believed to be a potential contributor in the antimicrobial resistance (AMR) in aquatic environments. The aim of this study was to describe the temporal dynamics of the resistome, mobilome, and virulome of a eutrophic river system.

Procedure/Method: We analysed the pre, during and post-eutrophication river samples using chemical, microbiological and molecular techniques, including metagenomics, whole-genome sequencing (WGS), and Real-Time PCR.

Findings/Results: The abundance of antibiotic resistance genes (ARGs) was altered one week after the eutrophication, returning to pre-eutrophication levels by week eight. The levels of clinically relevant β-lactam (blaCTX-M, blaSHV, blaGES, blaAIM1), colistin resistance (mcr1 and mcr4), and tigecycline resistance (tetX) genes significantly increased during the eutrophication. A rise in the abundance of imipenem-hydrolyzing β-lactamase gene (blaIMI) correlated with the increased levels of Enterobacter spp. post-eutrophication. Furthermore, the levels of the class 1 integron-integrase gene (intI1) and insertion sequences (IS26, IS1071) significantly increased within the first week of eutrophication, declining to near baseline levels thereafter. The abundance of clinically relevant virulence-associated genes (VAGs) associated with high-priority pathogens Corynebacterium diphtheriae, Salmonella enterica, and Edwardsiella tarda peaked after the eutrophication. Finally, WGS found unknown virulent and drug resistant Luteimonas spp., Stenotrophomonas spp., and Pseudomonas spp. that were not present in the pre-eutrophication samples.

Implications/Applications: Eutrophication-induced changes in AMR, mobile genetic elements (MGEs) and VAGs determinants in river systems are significant, suggesting that a eutrophic river environment may promote the development and survival of drug-resistant virulent opportunistic bacteria. These findings highlight the importance of managing eutrophication and its associated impacts to mitigate the spread of AMR and reduce water-related diseases and associated health risks.

Author: Mira Cooper-Beknazarova, University of South Australia, Australia

Co-Authors: Opoku, Solomon; Donner, Erica; Wyrsch, Ethan; Cummins, Max; Djordjevic, Steven; Vasileiadis, Sotirios; Awad, John; Chow, Christopher; Saint, Christopher; Drigo, Barbara

Session A

Abstract 306

Poster Board number: 24

Clinical class-one integron recombination is linked to prokaryotic stress response during conventional biological wastewater treatment

Background and Aim: Clinical class 1 integron-integrases (CIs) are mobile genetic elements (MGEs) capable of sampling and expressing antimicrobial resistance genes (ARGs) from the environment. CIs are one of the most important MGEs because they frequent human pathogens and can facilitate antimicrobial resistance (AMR) to all known classes of antimicrobials, posing a significant risk to public health. The integrase is encoded by intI1 whose transcription is regulated by prokaryotic stress response (i.e., the SOS response), which is triggered by DNA damage to the cell. While much is known about CIs in model bacteria, their role in wastewater microbiomes, when stressed, is largely unstudied. The purpose of this study was to measure prokaryotic stress response and CI expression through wastewater treatment processes and sequence full-length CIs and their transcripts to observe CI recombination events “in real time.”

Procedure/Method: We sampled three wastewater treatment plants with common biological treatment trains that discharged directly to surface waters to track CI dynamics. We then used tandem DNA/RNA extraction techniques and (RT)-ddPCR to quantify intI1, recN (site specific recombination), dinB (DNA polymerase IV), and sfiA (arresting cellular division) relative expression levels. Finally, we sequenced full-length CI mosaics and gene cassette transcripts using a MinION.

Findings/Results: Relative intI1, recN, dinB, and sfiA expressions levels (transcripts/total gene pool) steadily increased at each stage of treatment and were highest in receiving river water. Relative intI1 expression was directly correlated with relative SOS response gene expression. Of the total CI gene cassette pool (genomic DNA), 12% were being actively transcribed, less than 1% of which were identified as being ARGs.

Implications/Applications: This study provides new insights into resistome dynamics within wastewater environments. The methods/procedures described herein can be used to better understand engineered wastewater systems to control/mitigate the evolution of AMR.”

Author: Benjamin Davis, Jay Garland, U.S. EPA, United States

Back to the Program

Session A

Abstract 353

Poster Board number: 25

Impact of simple versus complex wastewater compositions on the fate of antimicrobial resistance genes in activated sludge wastewater treatment

Background and Aim: Wastewater treatment plants are propagators of Antimicrobial Resistance Genes (ARGs) in the environment. However, their complex dynamics in activated sludge remain unexplained. The present study investigated the fate of ARGs immigrating from upstream sewer into activated sludge reactors systems by manipulating the compositions of synthetic wastewaters from simple to complex to determine their impacts on the persistence of different ARGs.

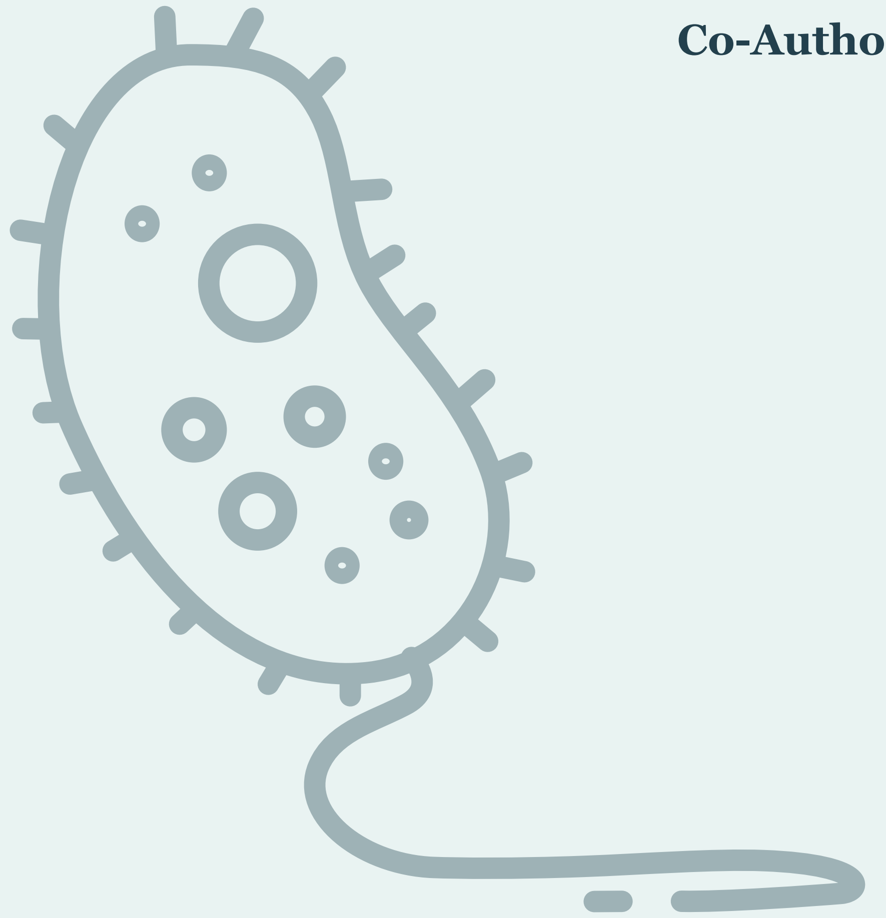
Procedure/Method: Erlenmeyer flask reactors received synthetic wastewater of two different compositions: one rich in sugars and volatile fatty acids (i.e., simple and readily biodegradable), the other rich in proteins and lipids (i.e., complex and slowly biodegradable). Full-scale influent solids were supplied to simulate immigration from upstream sewer systems. Amplicon sequencing of the 16S rRNA gene resolved the microbial community dynamics, and qPCR and multiplex amplicon sequencing determined the ARGs' abundance and diversity.

Findings/Results: Microbial communities that received the more complex, slowly degradable wastewater were more diverse and exhibited higher immigration rate than communities that received the simpler, readily degradable wastewater. A total of 81 ARGs were detected by qPCR in the sludges, and complex and simple feeds differed in the abundances of a minority of these ARGs (9 and 17 ARGs, respectively) regardless of immigration. ARGs related to the aminoglycoside and quinolone family were particularly increased by the complex feed. Immigration significantly increased the concentrations of 8 genes (compared to sludges with no immigration). Out of those 3 of them were significantly higher for the complex feed and only one for the simple feed.

Implications/Applications: This study suggests that the chemical wastewater composition and immigration influence the prevalence of ARGs carried by activated sludge communities, where complex and slowly biodegradable substrates enhance the presence and diversity of ARGs. This means that a greatly polluted wastewater poses a high risk of spreading antimicrobial resistance in the environment.

Author: Emmanuel Diaz Mendoza, McGill University, Canada

Co-Authors: Gibson, Claire; Kramer, Susanne; Frigon, Dominic



Session A

Abstract 278

Poster Board number: 28

'Resistome' of Kettle Lake in Ontario, Canada Includes 16S and 23S rRNA Mutations Conferring Antimicrobial Resistance

We investigated the antimicrobial resistance genes or 'resistome' in a kettle lake sample to characterize the mechanisms present that permit bacteria to resist antibiotics present in its environment. Antimicrobial resistance mutations in the 16S and 26S ribosomal RNA have been previously characterized in species such as Escherichia coli, Neisseria meningitidis, and Salmonella, and confer resistance to antibiotics. We performed shotgun metagenomic sequencing using Oxford Nanopore Technology, GridION, on the kettle lake sample from Lake Wilcox in Ontario, Canada (43° 56' 59.1" N -79° 26' 11.5" W). Barcode and adapter sequences were trimmed, and sequence quality was assessed with Fastqc. Identification of antibiotic resistance genes was performed with a KMA alignment against the Comprehensive Antibiotic Resistance Database (CARD). We found antibiotic resistance mutation in the 16S rRNA gene rrsA conferring resistance to kanamycin, a 23S rRNA mutation conferring resistance to clindamycin and a 16S rRNA mutation in the rrsD gene conferring resistance to spectinomycin. Only those sequences with 80% identity and 90% coverage with kept for analysis. The antibiotic resistance genes found were not associated with any known phage or plasmids by using Phaster and PlasmidFinder, respectively. Additionally, we performed taxonomic classification using a database of known marker genes and found that the antibiotic resistance genes were not specific to any known bacterial species suggesting that there may be species yet to be discovered in this kettle lake system. Given that Wilcox lake is a closed system but still harbouring mechanisms of antibiotic resistance with similarity to those found in known human pathogens is notable. The antibiotic resistance mutations detected in 16S and 23S rRNA is noteworthy as to date these findings have not yet been reported in an environmental sample.

Author: Anastasia Fedynak, University of Guelph, Canada

Co-Authors: Bryan, Noah; Soni, Mitra Soni; Parreira, Valeria; Precious, Melinda; Lawal, Opiyemi; Chen, Yanhong; Goodridge, Lawrence

Session A

Abstract 21

Poster Board number: 29

Antibiotic resistome beneath the surface: River biofilms reveal selection pressure of environmentally relevant concentrations of antibiotics.

Freshwater environments are the primary receiving systems of AMR reservoir “hot spot” run-off, such as wastewater treatment plant (WWTP) and agriculture effluents. Thus, AMR within aquatic environments may be driven by exposure of environmentally relevant concentrations of antibiotics (ERCA), below minimum inhibitory concentration (sub-MIC) and/or by the bacteria or resistance genes (ARG) present in various effluent. To determine whether exposure to ERCA or genetic material from AMR hotspots influenced the acquisition and proliferation of ARGs, we analysed structural and functional responses of exposed biofilm communities.

Riverine biofilms were developed in a microcosm system under constant exposure to an antibiotic cocktail (ciprofloxacin, oxytetracycline and streptomycin at 1/10 and 1/100 individual sub-MIC breakpoints), followed by supplementation with material from local AMR hotspots (WWTP and swine manure run-off). Confocal laser scanning microscopy (CLSM), metagenomic analyses, heavy metal and antibiotic residue analysis were performed to assess impacts of these treatments on biofilms. Metagenomics data was used to estimate the exposed biofilm resistome’s risk for AMR dissemination and potential human health risks through an environmental risk assessment (ERA) tool.

CLSM analyses demonstrated reduced production of extracellular polymeric substances in biofilm communities exposed to sub-MIC 1/10 antibiotic cocktail, suggesting weakened biofilms and increased bacterial cell sloughing. Metagenomic analyses revealed decreased taxonomic diversity, yet increased abundance and richness of ARGs and virulence genes after ERCA exposure, with significant co-occurrence of aminoglycoside and β-lactam ARGs to Pseudomonadota species. ERA risk scores indicated that ERCA exposure increased mobile genetic elements, with most ARGs (55 – 98%) classified as Risk Rank III and prevalent across all samples. Exposure to ERCA strongly influenced the biofilm resistome vs. exposure to AMR ‘hotspot’ material.

These observations support that riverine biofilms are reservoirs of AMR, enhancing and disseminating AMR-elements and thus environmental monitoring of antibiotic residues could identify priority areas for AMR/ARG surveillance.

Author: Gabriela Flores-Vargas, University of Saskatchewan, Canada

Co-Authors: Bergsveinson, Jordyn; Korber, Darren R.

Session A

Abstract 354

Poster Board number: 30

Antimicrobial resistance dissemination in hospital wastewater and associated aquatic environment in Joinville, Southern Brazil: A One Health approach

Background and Aim: Aquatic environments are increasingly impacted by contamination of anthropogenic origin, becoming an important source of antimicrobial resistance (AMR) genes dissemination. The “One Health” perspective aims to implement comprehensive policies in the fields of human, animal, and environmental health, including the prevention of AMR spread. In this context, a longitudinal investigation was performed to track the occurrence and dissemination of Gram-negative bacteria (GNB) carrying AMR genes in hospital wastewater (HWW) and associated aquatic environments.

Method: Samples of four HWW, downstream rivers and wastewater treatment plant (WWTP) were collected quarterly between October 2021 and August 2022, in Joinville, Southern Brazil. GNB were isolated and analyzed phenotypically and genotypically. Genes blaKPC, blaNDM, blaIMP, blaVIM, blaCTX-M and its 1, 2, 8 and 9 subgroups, blaSHV, blaTEM, mcr (1, 2, 4 and 5), blaOXA-23, blaOXA-24, blaOXA-51, blaOXA-58, and blaOXA-143 were investigated by Polymerase Chain Reaction. Furthermore, Pulsed-field Gel Electrophoresis (PFGE) was performed on Escherichia coli and Klebsiella pneumoniae isolates showing similar phenotypic and genotypic profiles. Results: Ninety-nine GNB were identified and the majority (70.7%) presented at least one of the AMR genes investigated. Samples of HWW showed the highest prevalence of AMR genes (65.7%). The gene blaCTX-M was the most frequent (58.6%), followed by blaKPC (45.7%) and blaTEM (47.1%). Coexistence of AMR genes was identified in 67.1% of isolates. The PFGE-based phylogenetic analysis showed four and six phylogroups for E. coli and K. pneumoniae, respectively, including isolates from the same phylogroup at different sampling points.

Implications: Of note, HWW was confirmed as an important source of aquatic dissemination of GNB carrying AMR genes, impacting an ecosystem where more than a million inhabitants live. In addition, a high phylogenetic variety and wide distribution of AMR genes were observed, which highlights the need for permanent surveillance and recovery of environments affected by hospital activities.

Author: Paulo Franca, University of Joinville Region - Univille, Brazil

Co-Authors: Correa, Ana; Kobs, Vanessa; Ferreira, Leslie; Sincero, Thais; Oliveira, Therezinha

Back to the Program

Session A
Abstract 345

Poster Board number: 31

Antimicrobial resistance and antimicrobial residues in the different stages of commercial poultry environment and across the food chain

Background and aim: Antimicrobial resistance (AMR) threatens the poultry industry, causing economic losses due to increased mortality rates and higher treatment costs. The objectives of this study were i) to determine AMR levels in the environment of different stages of commercial poultry farms; ii) to quantify antimicrobial residues present in the same environment; iii) to determine impact of management practices and antimicrobial residue on AMR.

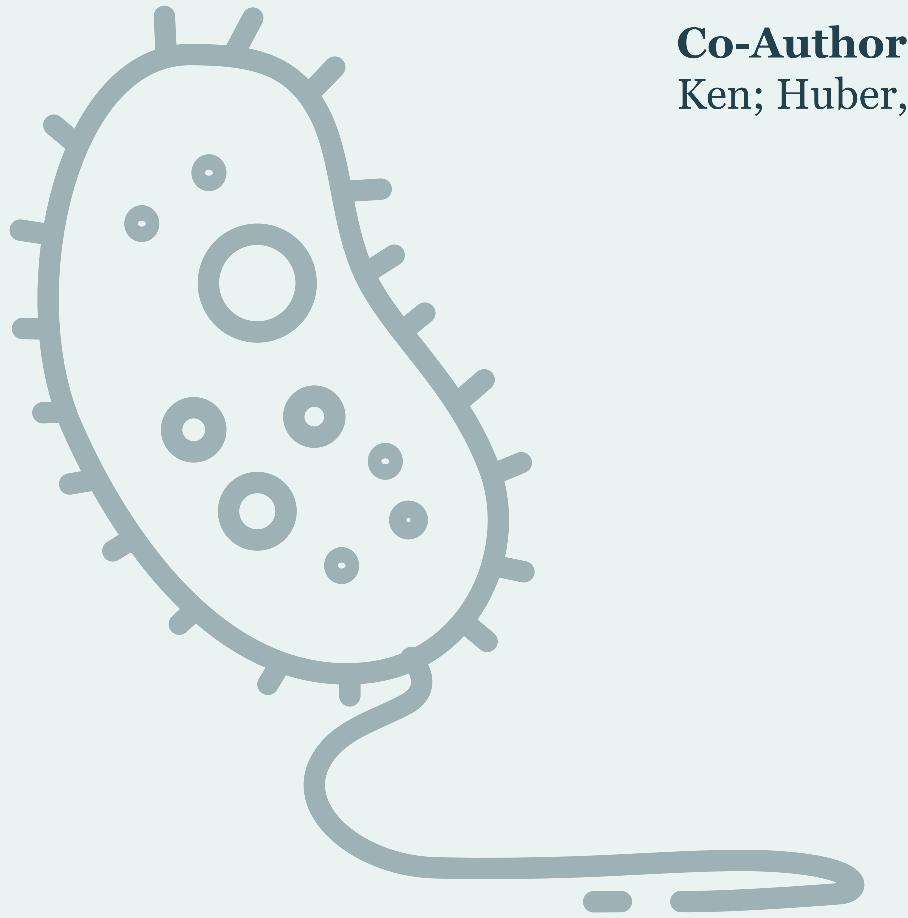
Methods: Commercial poultry farms in different stages of production practicing restricted antimicrobial use were included (N=16). Data on farm practices, antimicrobial use (AMU), and samples from litter, soil, and carcass rinses were collected. Frequency of 3 mobile genetic elements (MGE) and 14 antimicrobial resistance genes (ARGs) conferring AMR to 8 antimicrobial classes was assessed using qPCR. Liquid Chromatography-Mass Spectrometry will be used to detect antimicrobial residues.

Results: Poultry farms in this study had history of AMU for therapeutic purposes. In broiler farms, frequency of AMR to most antimicrobial classes was higher than breeder and pullet farms. Frequency of sulfonamide ARGs was higher in pullet and breeder farms, while beta-lactam ARGs were more common in breeder farms. AMR to most antimicrobial classes was higher in litter than soil. In processing plant, most ARGs were found only in post-pick stage except that aminoglycoside ARGs were also found in post-chill stage. MGEs were most predominant on broiler farms, litter, and post-chill stage. Additionally, impact of antimicrobial residues and management practices on AMR will be presented.

Implications: Despite restricted AMU, there is potential for AMR spread across the food chain in commercial poultry. Historic AMU may have resulted in the persistence of antimicrobial residue in environment of farms with restricted AMU. Understanding the impact of historic AMU on AMR and accumulation of antimicrobial residues in the environment will provide potential pathways of AMR transmission across the food chain.

Author: Pankaj Gaonkar, Dept. of Pathobiology, Auburn University, United States

Co-Authors: Golden, Reed; Higgins, Courtney; Bailey, Matthew; Adhikari, Yagya; Macklin, Ken; Huber, Laura; Boersma, Melissa



Session A
Abstract 275

Poster Board number: 32

The Wastewater Resistome: a 29-month survey of Quebec City wastewater metagenomes reveals plasmids as drivers of emergent antimicrobial resistance

Background and Aim: A change in paradigm for pathogen surveillance is the use of metagenome assemblies from complex communities using long-read DNA sequencing. In this sense, wastewater is an excellent biomarker as it is an abundant byproduct of human populations and their gastrointestinal microbiota.

Method: Wastewater samples from Quebec City obtained weekly were sequenced using the Oxford Nanopore GridION and PromethION2 platforms, each giving a per-week output of 20-25 Gigabases of DNA data (GridION, from Oct 2021 to Feb 2023) and 150-200 Gigabases (P2 solo PromethION2, from July to Nov 2023). Metagenomes were then reconstructed by de novo assembly (metaFlye v2.9) and taxonomic assignment with Kraken2 v2.1.2. AMR genes were predicted with RGI v6.0.2 using the most recent version of the Comprehensive Antibiotic Resistance Database (CARD).

Results: A total of 970,281 metagenomic DNA fragments (contigs) were reconstructed from this 27-month survey. From those, 22,294 AMR genes were detected with 692 being plasmid-mediated. The top taxa harboring plasmid-mediated AMR were Acinetobacter, Aeromonas, Acidovorax, Bacteroides, Escherichia, Klebsiella, Pseudomonas and Salmonella spp. For all these except Acidovorax and Bacteroides, genes involved in inactivation of carbapenems and aminoglycosides were found in higher prevalence in the fall season; these include blaGES, blaOXA (carbapenem); AAC, ANT and APH (aminoglycoside). Conversely, qnr (fluoroquinolone) and sul (sulfonamide) were found in high abundance during the summer season. Besides, 74 other genera contributed to AMR for 16 other antibiotics classes.

Applications: This work illustrates a method to continuously monitor AMR in urban communities through metagenomic sequencing of wastewater effluents. Furthermore, a single PromethION2 flow cell provides as much sequencing throughput than 5 standard MinION flow cells for 2.5 fewer sequencing costs. This disruptive culture-independent method could allow health authorities to establish early warning systems to monitor antimicrobial resistance (AMR) emergence and horizontal transfer to and from the human gut microbiota.

Author: Jeff Gauthier, Université Laval, Canada

Co-Authors: Potvin, Marianne; Mohammadi, Sima; Kukavica-Ibrulj, Irena; Renaud, Valentine; Quang Henri Nguyen, Guillaume; Landgraff, Chrystal; Nichani, Anil; Goodridge, Lawrence; Boyle, Brian; Levesque, Roger C.

Back to the Program

Session A

Abstract 352

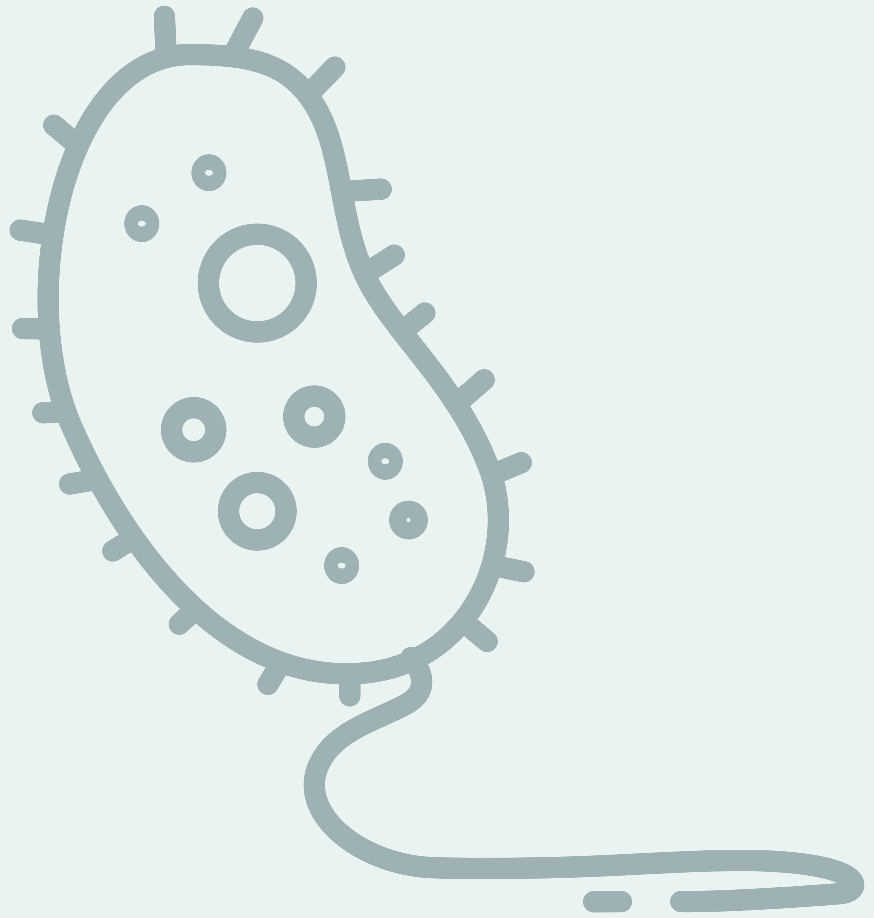
Poster Board number: 33

Characterisation of antimicrobial-resistant Escherichia coli isolated from retail chicken breasts

Antibiotics are used in animal husbandry to increase productivity. However, excessive use contributes to the development of antimicrobial resistance (AMR) in bacteria. Livestock and poultry products are now AMR reservoirs. We isolated bacteria from 60 samples of chicken breasts sold in Canada in 2016. Escherichia coli was predominant. We are interested in the AMR profiles and pathogenic potential of the E. coli isolates. The E. coli isolates were isolated by using enrichment broths to select Enterobacteriaceae then selective agars for chromogenic differentiation of E. coli isolates. We then used a MALDI-TOF mass spectrometer for taxonomic classification. Biofilm morphotype was determined by spotting on Congo Red Coomassie Blue agar plates, and biofilm production was measured using microtitre plate assays. Antimicrobial susceptibility profiles were determined using broth microdilution assays. PCR analysis confirmed the presence of third-generation cephalosporin resistance genes. Illumina MiSeq sequencing and bioinformatic analyses revealed the sequence types, resistance profiles, mobilome, virulence factors and serotypes. Most isolates were resistant to at least four of the following antibiotics: cefoxitin, ceftriaxone, tetracycline, ampicillin, streptomycin, gentamicin, or chloramphenicol. Forty-one isolates carried the CMY-2 gene, while other AMR genes were less common. Eighteen isolates exhibited extended-spectrum beta-lactamase activity. Forty-four E. coli isolates formed biofilms of the red dry and rough morphotype. We discovered isolates that contained multiple plasmids carrying virulence factors and AMR genes. The biofilm results are concerning because biofilms decrease susceptibility to antibiotics and disinfectants. In addition, AMR genes were present in many mobile genetic elements that may be transferable. The presence of virulence factors indicated that some isolates may be pathogenic. Our results show the extent of antibiotic resistance in chicken meat and exemplify the importance of mitigating antibiotic resistance in poultry.

Author: Paul Glenn, McGill University, Canada

Co-Authors: Ronholm, Jennifer; Tamber, Sandeep; Rao, Mary



Session A

Abstract 402

Poster Board number: 34

Understanding roles of non-antibiotic pharmaceuticals on the emergence and transmission of antibiotic resistance

Although non-antibiotic pharmaceuticals are widely used by society with 95% of the drugs market share, little is known about whether non-antibiotic pharmaceuticals cause or accelerate the dissemination of antibiotic resistance. We have been focusing on exploring the interactions between non-antibiotic pharmaceuticals and the transmission of antibiotic resistance in the last five years. In the lab, we established multiple bacterial models, including mutation, conjugation, transformation, and in vivo animal gut microbiota assays to investigate antibiotic-like effects of commonly-used non-antibiotic drugs (e.g. antidepressants, anti-inflammatories and lipid-lowering drugs) on the emergence and spread of antibiotic resistance. We found that these non-antibiotic pharmaceuticals not only induce the emergence of antibiotic resistance through mutation, but also promote the dissemination of antibiotic resistance via conjugation and transformation. This was demonstrated in both pure-culture bacterial models and mixed-culture environmental samples, as well as animal gut microbiota. Several common mechanisms, including over-generation of reactive oxygen species, cell membrane variation, and stress level elevation, are playing key roles. Considering the high-consumption and wide-detection of non-antibiotic pharmaceuticals, these findings emphasize concerns of non-antibiotic pharmaceuticals for the emergence and spread of antibiotic resistance in the environment and advance our current understandings of the dissemination of antibiotic resistance. We would like to share our findings and discuss research questions that entail further studies in this talk.

Author: Zhigang Yu, The University of Queensland, Australia

Co-Authors: Guo, Jianhua; Wang, Yue; Ding, Pengbo; Lu, Ji

Back to the Program

Session A

Abstract 16

Poster Board number: 35

Chlorination stress can promote horizontal transfer of antibiotic resistance genes within mixed-culture biofilms

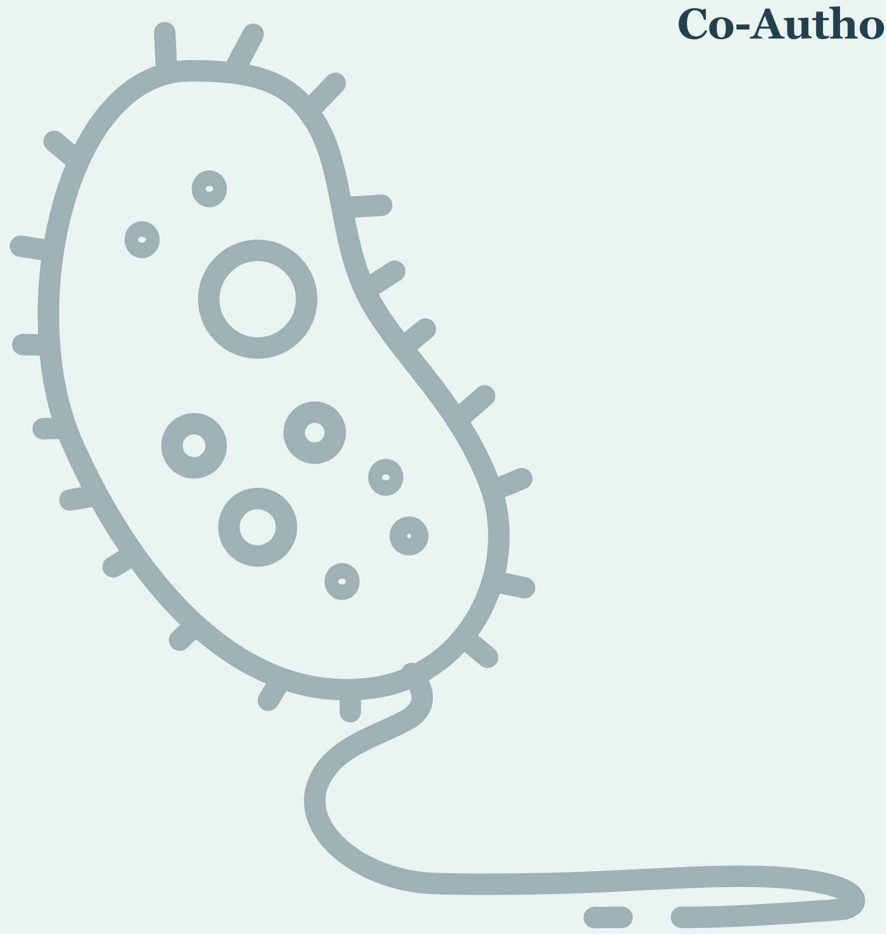
Background and Aim: Chlorination disinfection is widely applied to control microbial contamination in drinking water pipeline systems. Existing studies have reported the abundance of antimicrobial resistance (AMR) in water distribution systems, but little is known about AMR dissemination in biofilms, in which form more than 90% of the biomass resides in water sources. This study aims to comprehensively investigate the side effects of chlorine in the spread of antibiotic resistance in biofilms from water system.

Procedure/Method: Both pure and mixed culture biofilms (including Escherichia coli, Pseudomonas putida, Pseudomonas aeruginosa, and their combinations) were established, with pKJK5-carrying E. coli used as the donor. After exposure to serial concentrations of free chlorine, the conjugative transfer was analysed by antibiotic selective plates and microscope imaging. CDC biofilm reactors were also used to mimic the real scenario in the drinking water distribution systems. The underlying mechanisms were revealed by analyses, including oxidative stress, cell membrane permeability, and whole-genome DNA/RNA sequencing.

Findings/Results: Our results demonstrate that at environmentally relevant concentrations (e.g., 5 mg/L), free chlorine significantly promotes conjugative transfer (e.g., around 26-fold increase in conjugation ratio) of plasmid encoded antibiotic resistance genes (ARGs) among both pure and mixed biofilm bacteria. We find that chlorine destructs the recipient biofilms and enables the donor to colonise the recipient biofilms. However, ARG transfer only occurred at the surface or outer layer of recipient biofilms. Chlorine-mediated conjugative transfer is due to excessive oxidative stress, increased membrane permeability, and the provoked SOS response from both donor and recipient. Expressional levels of genes responsible for ARG transfer process were significantly upregulated during chlorine exposure. Implications/Applications: Our findings offer insights into the chlorine disinfectant associated AMR spread in the drinking water distribution system and provide evidence for the development of novel strategies to mitigate AMR issue and secure water quality.

Author: Zhingag Yu , The University of Queensland, Australia

Co-Authors: Li, Yujie; Guo, Jianhua



Session A

Abstract 264

Poster Board number: 36

A nanopore long-read sequencing points out a modification of hospital wastewater microbiome and resistome after chlorine disinfection

Hospital wastewater (HWW), discharged from operating rooms, laboratories, and infectious wards contains pathogenic microorganisms, toxic organic pollutants, and pharmaceutical compounds such as antimicrobials and psychiatric drugs. The presence of numerous pathogenic bacteria in HWW highlights the potential threat to public health posed by HWW discharge to municipal sewer systems followed by wastewater treatment plants and receiving water. To reduce that risk, HWW is often treated in local disinfection stations. Chlorine-based wastewater disinfection is widely used around the world. The aim of this study was to analyze changes in bacterial communities and specific antibiotic resistance genes (ARGs) in HWW with the use of a nanopore long-read metagenomic approach. Furthermore, the main hosts of ARGs in HWW were identified, and the mobility of resistance mechanisms was analyzed. The study was conducted in a hospital that treats patients with respiratory diseases in north-eastern Poland. Wastewater is disinfected by an automated electrolytic sodium hypochlorite (NaClO) generation system. Samples of wastewater before disinfection and samples of disinfected wastewater were analyzed. The results of this study indicate that chlorine disinfection of HWW can induce significant changes in the structure of the total bacterial population and antibiotic resistant bacteria (ARB) communities, and that it can modify the resistome and mobilome of HWW. Disinfection favored the selection of ARGs, decreased their prevalence in HWW, while increasing their diversity. The mobility of the HWW resistome increased after disinfection. Disinfection led to the emergence of new drug resistance mechanisms in previously sensitive bacterial taxa. In conclusion, this study demonstrated that HWW disinfected with low (sublethal) concentrations of free chlorine significantly contributes to the mobility and transfer of drug resistance mechanisms (including critical mechanisms) between bacteria (including pathogens). Acknowledgements: This research was funded by grant from the National Science Center (Poland) No. 2021/41/N/NZ9/03292.

Author: Monika Harnisz, University of Warmia and Mazury, Poland

Co-Authors: Rolbiecki, Damian; Paukszto, Łukasz; Sawicki, Jakub; Korzeniewska, Ewa

Back to the Program

Session A

Abstract 166

Poster Board number: 37

Fate of antibiotic resistance genes and mobile genetic elements during mesophilic anaerobic digestion and post treatment of digestate in french farms.

Background and aim: On farm mesophilic anaerobic digestors are increasingly used for treating animal and crop wastes (manures) and to produce renewable energy. Manures can be contaminated with AMR genes and mobile genetic elements, and antibiotic residues. Here, we evaluated the fate of several clinically relevant AMR genes and mobile genetic elements during the process of anaerobic digestion, dewatering of digestate, and composting of digestate solids. Three on farm digestors were studied.

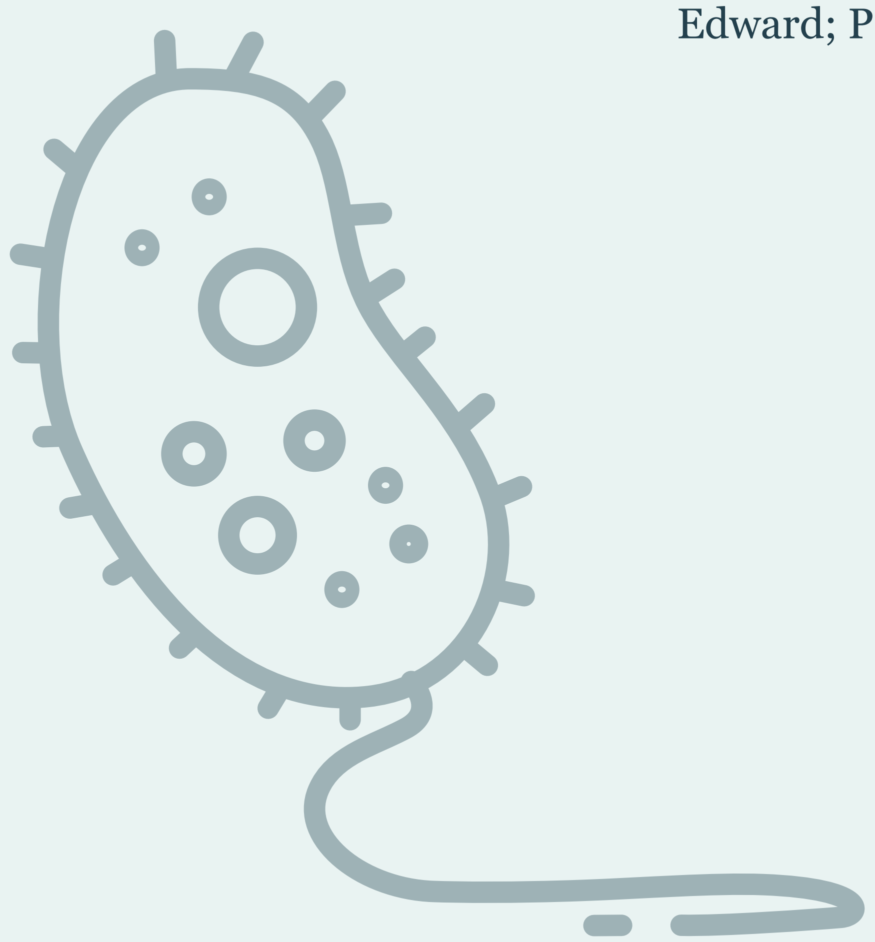
Methods: Triplicate samples from the different stages of the process (including initial input material) were collected six times over a 15 months period. DNA of samples were extracted and Taqman real time PCR was used to quantify the gene targets sul1, sul2, tetA, tetM, blaCTX-M group 1, blaCTX-M group 9, qnrD, mcr1, intI1, intI2, korB, and qacE. Gene copy numbers were normalized to total 16S rRNA gene. A non-parametric variance partition analysis was carried to evaluate the importance of sampling time, the sampled matrix, and their interaction on the evolution of the gene quantifications at each farm.

Results: After anaerobic digestion, we observed a decrease of most target gene ratios (1 to 2 fold) compared to the input material. However, the AMR copy number ratios did increase in the composted solid phase of digestate or liquid fraction of digestate. Composted digestate contains higher copy number gene ratios that the digestate itself for some target genes.

Implication: Anaerobic treatment of animal manure leads to the production of digestates that still carry AMR genes at high levels (up to 109 copies per g of dry matter for sul and tet genes), at moderate level (103 to 105 copies per g of dry matter for qnrB, mcr1 and blaCTX-M genes). Land application of these post-treated digestates might contribute to the environmental dissemination of AMR in the same way as digestates themselves

Author: Alain Hartmann, INRAE, France

Co-Authors: Depret, Geraldine; Druilhe, Céline; Jacquiod, Samuel; Roux, Apolline; Topp, Edward; Pourcher, Anne-Marie



Session A

Abstract 61

Poster Board number: 38

The selective effects of commonly used non-antibiotic pharmaceuticals in combination with ciprofloxacin

Background and Aim: The aquatic environment is polluted with antibiotics and non-antibiotic drugs (NADs). The selective effects of antibiotics on antimicrobial resistance (AMR) are of concern. Mixtures of antibiotics and NADs are less well studied, yet they may still select for AMR. The aim in this study was to identify the effects of three NADs alone, and in combination with ciprofloxacin, on complex bacterial community growth, and selection for an AMR marker (intI1).

Procedure/Method: A wastewater influent community was used to investigate the effects of diclofenac, metformin, and 17-β-estradiol, individually, and in pair-wise mixtures with ciprofloxacin. A concentration gradient of single NADs were tested to determine effect concentrations on growth. Then, NADs were added as ‘spiked’ single concentrations along a ciprofloxacin concentration gradient. Changes in growth of the community were measured with optical density over a 12 hour period. Additionally, seven day selection experiments (inoculation into media spiked with ciprofloxacin, NAD, or ciprofloxacin and NAD, with daily transfers) were performed with changes to intI1 molecular prevalence quantified using qPCR.

Findings/Results: All NADs significantly reduced growth of the community (diclofenac from 50µg/L, metformin from 25.781µg/L, and 17-β-estradiol from 24.375µg/L), but only 17-β-estradiol also selected for intI1 (from 7µg/L to 5400µg/L). Ciprofloxacin reduced the growth of the community (from 1.95µg/L) and selected for intI1 at 40µg/L. However, presence of all three NADs increased the activity of ciprofloxacin, reducing the lowest observed effect concentration against growth. Additionally, presence of all three NADs alongside ciprofloxacin reduced the concentration at which intI1 was selected for (from 40µg/L to 10µg/L).

Implications/Applications: These data show that even non-selective pharmaceuticals can increase the selective activity of antibiotics, indicating that we may be underestimating the risk of AMR within the aquatic environment. Future studies should include non-antibiotic environmental pollutants when investigating selection and maintenance of AMR by complex mixtures.

Author: April Hayes, University of Exeter, United Kingdom

Co-Authors: Zhang, Lihong; Feil, Edward; Snape, Jason; Kasprzyk-Hordern, Barbara; Gaze, William; Muuray, Aimee

Back to the Program

Session A

Abstract 46

Poster Board number: 39

Tracking antibiotic resistome structure and its driving factors from Qiantang River Basin to the downstream Hangzhou Bay

Background and Aim: Antibiotic resistance genes (ARGs) have amplified microbial threats to human health through horizontal gene transfer, circulating among human, animals, and environment. Hangzhou Bay, which connects terrestrial and marine ecosystems, serves as a hotspot for antimicrobial resistance. Therefore, a comprehensive understanding of antibiotic resistome is the cornerstone of understanding and controlling the ARGs risk in Hangzhou Bay from the perspective of 'one health'.

Procedure/Method: This study utilized metagenomics to analyze ARGs in the sediments of Hangzhou Bay and its upstream Fuchun and Qiantang rivers (as drinking water sources) and assess their risks by weighted summation of different risk rankings of ARG abundance. Furthermore, the impact of environmental and biotic factors on the resistome structure was evaluated.

Findings/Results: Both the abundance of ARGs and resistome composition shifted across the examined watersheds. The total abundance of ARGs (copy/cell) generally increases from Funchun river (1.72-2.35×10-3) to Qiantang river (4.33-7.38×10-3) and then decreases along the river flow into HZB (2.01-6.30×10-3). Bacitracin, sulfonamide, and macrolide-lincosamide-streptogramin resistance genes are the predominant ARGs in Fuchun river. In contrast, beta-lactam, bacitracin and novobiocin resistance genes are prevalent in the downstream watersheds. The ARG risk increase in HZB is caused by the enrichment of clinically relevant streptogramin resistance genes (vatE) mostly hosted by nitrifying bacteria Nitrospirae. Water quality parameters examined (18.5%) uniquely best explained the variation of HZB resistome, followed by microbiota composition (15.9%) and pharmaceutical and personal care products (7.8%).

Implications/Applications: This study improves the understanding of resistome connectivity between Hangzhou Bay and upstream Qiantang Basin and provides a scientific basis for further improving measures to evaluate and mitigate antibiotic resistance risks along this coastal city waterway. The enrichment of clinically relevant ARGs in environmental nitrifying bacteria in Hangzhou Bay reveals the potential connection between environmental and clinical resistance. More exhaustive studies are thus necessary.

Author: Xinyu Huang, Westlake University, China

Co-Authors: JU, Feng

Session A

Abstract 343

Poster Board number: 40

Antimicrobial Resistance in Wild Escherichia coli Isolates from Whitetail Deer

Background and aim: Antimicrobial resistance (AMR) is a major global public health threat. AMR organisms (AROs) and genes (ARGs) are now widespread in humans, the environment, and in wild and domestic animals. The transmission of AROs and ARGs from humans to the environment happens through various means such as contamination of river and lake waters, transmission through sewage waters, and the transmission of mobile genetic elements carrying AMR determinants between humans and wild animals. Our objective is to study AMR transmission and resistance patterns in Escherichia coli derived from whitetail deer.

Procedure/method: 40 isolates derived from the guts of white-tailed deer were obtained from the Penn State University E. coli reference center. Following shotgun genomic sequencing, in silico analysis was performed to detect resistance genes, plasmids, and other resistance determinants. AMR was measured using minimum inhibitory concentration (MIC) assays towards ampicillin, streptomycin, ciprofloxacin, tetracycline, and ceftazidime. Phylogenetic analyses were used to identify possible transmission patterns of AMR plasmids.

Findings/results: Resistance to ampicillin, streptomycin and ciprofloxacin was recorded based on CLSI cutoffs. Resistance genes for a variety of antimicrobials were detected, and largely predicted AMR phenotypes. Several deer-derived AMR plasmids were nearly identical to those from human clinical isolates.

Implications/applications: This study will pave the way for a better understanding of the patterns, transmission, and spread of AMR in wild E. coli isolates. Close relationships between deer-derived resistance plasmids and human-derived sequences suggests recent inter-species transmission. In addition, potential resistance mechanisms can be inferred from MIC correlations between drugs.

Author: Asalia Ibrahim, Carleton University, Canada

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 383

Poster Board number: 41

The impact of nitrogen fertilization on antimicrobial resistance genes in Rhizobium leguminosarm trifoli.

Background & aim: The phytobiome, soil microbes that colonize plant tissue, play an important role in plant health and ecosystem stability. Rhizobia are members of legume plant phytobiomes that perform nitrogen fixation, making them agriculturally relevant. The composition and function of phytobiomes can be shaped by antimicrobials. Antimicrobials are normally produced by microbes but may also be introduced through agricultural runoff. Being a strong selective force, antimicrobials drive the rapid evolution of antimicrobial resistance via selection of antimicrobial resistance genes (ARGs). Sequencing technologies can be used to investigate the diversity of ARGs within a phytobiome which would be powerful for predicting the impact antimicrobials have on them, and in turn, plant health.

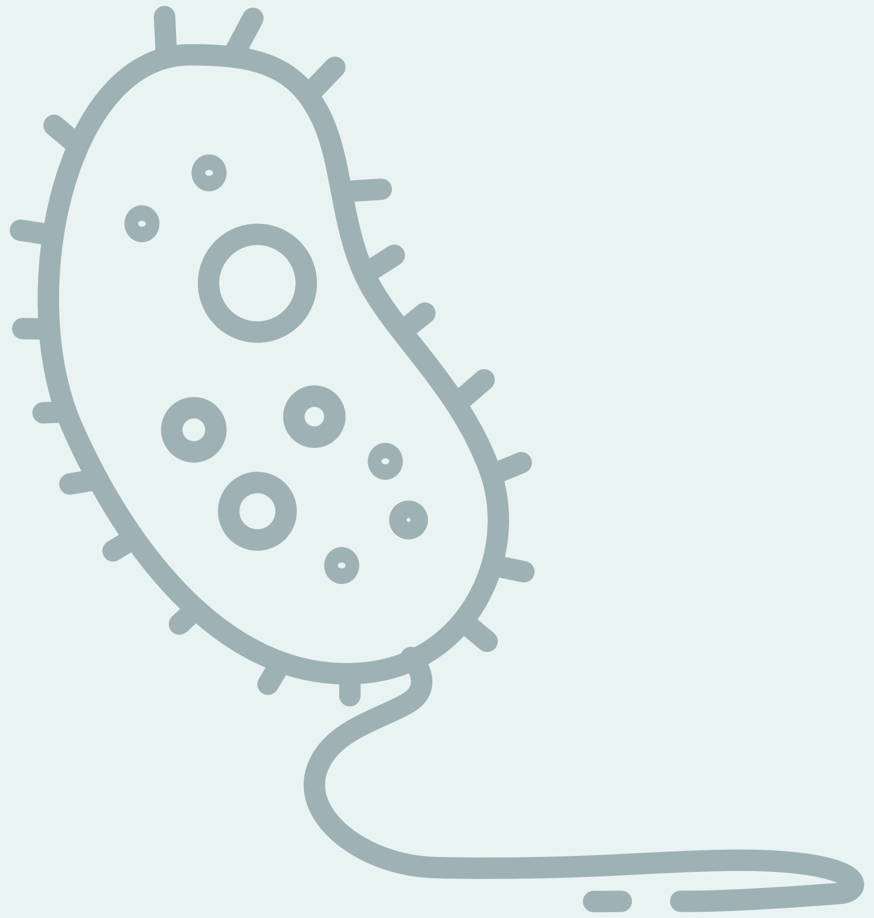
Procedure & methods: ARGs were identified from 56 Rhizobium leguminosarum trifolii strains that were either exposed to nitrogen fertilizer or not. The RGI and DeepARG programs were used to identify ARGs from long read datasets. The outputs from RGI and DeepARG were then compared to determine the relative abundance and diversity of ARGs from nitrogen-exposed strains to controls.

Findings & results: We hypothesize that strains exposed to nitrogen will have more ARGs and will be less beneficial plant symbionts (based on plant growth). By extension these strains will also have fewer genes related to nodule formation, given plants rely less on rhizobia when nitrogen is freely available in the environment.

Implications & applications: Through metagenomic sequencing, the diversity of ARGs within phytobiomes will be highlighted. The results will highlight whether N2 relates to an increases in ARGs and less fit Rhizobium strains. This allows for better prediction of the longer-term impact nitrogen fertilization will have on the health of ecologically and economically important crops as well as providing a better understanding of how nitrogen application will impact the outcomes of keystone plant-microbe symbioses.

Author: Isabella Ippolito, McMaster University, Canada

Co-Author: Doyle, Rebecca



Session A

Abstract 338

Poster Board number: 42

Mobility potential of latent antibiotic resistance genes

Background and aim: Many established antibiotic resistance genes (ARGs) have their origin in environmental microbes. The environmental pool of resistance determinants, latent ARGs, is larger both in abundance and diversity, than the established ARGs. These genes are often core genes of their hosts and require one or several mobilization events before becoming problematic ARGs. The mobility potential and host range of latent ARGs is mostly unexplored. Our aim is to predict mobility potential of latent ARGs and identify possible emerging resistance determinants in sewage. With host prediction we aimed to identify the most prominent bacterial hosts responsible for the emergence of new resistance determinants in sewage.

Method: We sampled one urban sewage and sequenced metagenomic DNA with long-read sequencing technology. HMM models were created for sulfonamide, trimethoprim and mobile colistin resistance genes based on established ARGs. Latent ARGs were searched with HMM models and known resistance genes removed. The mobility potential was determined with mobile element finder and plasmid reads identified with PlasX. Genome-resolved metagenomics and methylation profiles were used for host prediction.

Results: We identified a vast diversity of latent ARGs from urban sewage. Most of the latent ARGs were of chromosomal origin with no identified mobility potential. Latent ARGs with mobility potential included mobile colistin resistance genes residing in plasmids and several sulfonamide and trimethoprim resistance genes with mobile elements in proximity showing a potential for mobilization.

Implications: As horizontal transfer is the main driver behind the antibiotic resistance crisis and the environmental latent ARG pool provides wide diversity of novel resistance determinants, it is important to understand the mobility potential of these emerging ARGs to be able to consider their transmission risks. Identifying the hosts of latent ARGs with mobility potential can guide future mitigation efforts against the emergence of new antibiotic resistance determinants.

Author: Antti Karkman, University of Helsinki, Finland

Co-Authors: Markkanen, Melina; Dekić Rozman, Svjetlana; Muurinen, Johanna

Back to the Program

Session A

Abstract 417

Poster Board number: 43

Optimizing DNA extraction and sequencing approach for recovering ARG contextual information in wastewater

Background and Aim: Accurate and reproducible reconstruction of antibiotic resistance genes (ARGs) and their in-situ genomic contexts from environmental matrices is needed for in-depth monitoring and risk assessment of antimicrobial resistance (AMR). The purpose of this study was to systematically evaluate low- (LMW) versus high-molecular weight (HMW) DNA extraction techniques and combinations of sequencing (Illumina and nanopore) and bioinformatic techniques (single assembly, co-assembly, hybrid assembly) on the resulting assembly quantity (total assembly size) and quality (N50s, percentage of misassembles) of wastewater metagenomes. Further, the successful recovery of metagenome assembled genomes (MAGs) was evaluated based on the number, completeness, and contamination of bins to optimize genomic comparisons to clinical isolates.

Procedure/Method: We sampled a local wastewater treatment plant for influent, activated sludge, and secondary effluent samples and split each into 12 technical replicates (4 conditions in technical triplicate). Half were extracted using the FastDNA LMW kit, the other with the Zymo HMW kit. Each set of six were then sequenced on both an Illumina (NovaSeq) and ONT (PromethION) machine. Mock communities were also processed for each condition in technical triplicate. A custom bioinformatic pipeline was then used to evaluate pairwise combinations of assembly strategies for optimal recovery of ARG contextual information.

Findings/Results: We found significant differences in assembly quantity and quality between the 4 conditions (LMW+Illumina, LMW+ONT, HMW+Illumina, HMW+ONT) in both assembly quantity and quality based on both wastewater and mock community samples. The optimal combination for ARG contextualization was found to be HMW+Illumina data hybrid co-assembled with HMW+ONT data.

Implications/Applications: This study provides an optimized pathway for the recovery of high-quality metagenomic assemblies from wastewater. The techniques presented here can be extended to investigate other complex environmental matrices for AMR monitoring.”

Author: Chad Kelley, Virginia Tech, United States

Co-Author: Davis, Ben

Session A

Abstract 223

Poster Board number: 44

Flash Poster Presentation: s2 - Monday, May 27 - 8:30-10:15

Trimethoprim resistance in surface and wastewater is mediated by contrasting variants of the dfrB gene

Background and Aim: In a moderately polluted river, we found the abundance of all but one resistance gene family to be positively correlated with crAssphage occurrence. The one outlier was dfrB, a gene family providing resistance to trimethoprim. Inspired by this finding and the fact that novel variants of dfrB have recently been identified, we aimed at mapping the distribution of dfrB in aquatic systems to learn about the origin and spread.

Methods: We screened shotgun metagenomic sequences found in NCBI’s sequence read archive for all twenty variants of dfrB characterized so far, considering surface water, sediment, and wastewater samples (n=324, 7 countries). The flanking regions of dfrB sequences were assembled to unveil the genetic context. Relative abundance estimates were confirmed by qPCR.

Findings: In spite of high sequence similarity, the different variants of dfrB were found to be very unevenly distributed. Wastewater samples were dominated by the early discovered genes dfrB1 to dfrB7. Those variants were typically found in neighborship with other resistance genes and markers of mobility like insertion sequences and integrons. By contrast, river water and sediment samples were dominated by dfrB variants with a higher numeric index, dfrB10 being the most prevalent one (up to 1e-4 copies / 16S rRNA gene copies). This variant occurred in truly pristine places and no linkage with other resistance genes or mobile elements was detected. The original role of dfrB10 appears to be unrelated to resistance.

Implications: Aquatic systems were identified as a reservoir of novel dfrB variants not frequently seen in healthcare settings. However, they could gain clinical relevance after mobilization. In fact, mobilization has already been demonstrated, as dfrB10 was originally discovered on a plasmid harbored by a pathogenic Pseudomonas putida. We currently attempt to identify major environmental hosts of dfrB10 to eventually understand the bottlenecks in mobilization.

Author: David Kneis, TU Dresden, Institute of Hydrobiology, Germany

Co-Authors: Lemay-St-Denis, Claudèle; Cellier-Goetghebeur, Stella; Elena, Alan Xavier; de la Cruz Barron, Magali; Tskhay, Faina; Heß, Stefanie; Berendonk, Thomas U.; Pelletier, Joelle N.

Monday, May 27 - 16:00-18:00

Back to the Program

Session A
Abstract 347

Poster Board number: 45

Human and veterinary antibiotics use drives resistome diversity across hundreds of lakes

Background:. Lake environments integrate watershed processes and thus may act as receptors and reservoirs of antibiotic resistance genes (ARGs) introduced into the watershed by human activities, but previous research has been largely restricted to either a limited number of ARGs or lakes.

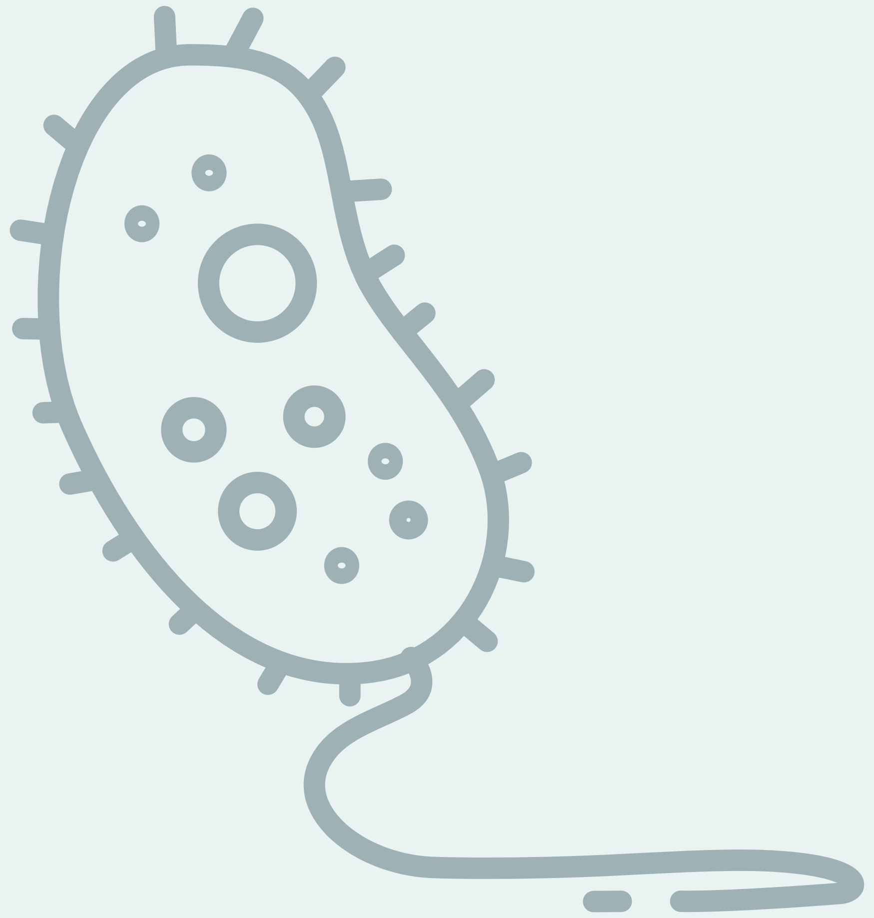
Methods: Here, we utilized metagenomics to assess the resistome of hundreds of Canadian lakes, located along a continuum of potential exposure to anthropogenic ARGs, including hospitals and wastewater treatment plants within the watershed. Secondly, we use meta-transcriptomics to track the activity of such genes in the environment for a subset of lakes.

Results: A high percentage of dtected ARG's presence was either unimpacted by human activity or highly prevalent in pristine lakes, highlighting the role of antimicrobial resistance genes (AMR) in microbial ecology. Specifically, these genes represent pools of ARGs available for potential horizontal gene transfer to current or novel waterborne pathogens. In addition to the identified 'natural' resistome, agricultural and pasture areas within the watershed were found to significantly impact resistance gene relative abundances. Point sources such as wastewater treatment plants and hospitals, as well as population density and the application of manure in the watershed were all found to significantly impact resistome diversity.

Implications: These findings indicate that lake environments are regularly stocked with resistance genes that continue to evolve in the context of both veterinary and human antibiotics use, and represent reservoirs of resistance genes that require further monitoring and clarification of priority targets.

Author: Susanne Kraemer, ECCC, Canada

Co-Authors: Broadbent, Jordyn; Walsh, David



Session A
Abstract 161

Poster Board number: 47

Abundance of antimicrobial resistance genes in clinically relevant bacterial pathogens isolated from a recreational freshwater lake in Ontario, Canada

Background and Aim: Antimicrobial resistance (AMR) is a global challenge that poses significant threats to public health. Aquatic ecosystems have the potential to act as reservoirs for resistance determinants to critically important antibiotics. Here, we assessed antimicrobial resistance genes (ARG) and their carriers in a recreational freshwater lake in Ontario.

Procedure/Method: Freshwater samples were obtained from a Kettle Lake in Richmond Hill, Ontario, Canada in the Summer of 2022 and 2023. Samples were preprocessed and plated onto tryptic soy agar (TSA). Colonies with different morphologies were characterized using VITEK® (bioMérieux, Inc, Canada). DNA from pure colonies was extracted and paired-end (2 x 150 bp) sequenced on the Illumina MiniSeq systems. Sequence reads were preprocessed and analyzed using bioinformatics tools.

Findings/Results: Sixteen isolates were recovered from the lake. Ten isolates were predicted as human pathogens and included six Aeromonas veronii and one isolate each for Acinetobacter sp., Escherichia coli, Staphylococcus aureus, and Vibrio cholerae. Resistome analysis revealed that fosB gene and point mutations (murA_D278E, murA_E291D, glpT_A100V, glpT_V213I, glpT_E448K) that encode resistance to fosfomycin were detected in S. aureus and E. coli. For ☐-lactam resistance, blaOXA was enriched (80%, n=13/16) in the collection. Others include cphA (Aeromonas spp.), blaEC (E. coli), blaCARB-7 (V. cholerae), blaPC1, blaR1, and blaI (S. aureus). Colistin resistance determinants (almEFG operon or pmrB_Y358N) were detected in Vibrio cholerae and E. coli. Resistance determinants for tetracycline (tet(38), mepA) and heavy metals (mco, and arsRBC) were carried by S. aureus. The chloramphenicol resistance gene (catB) was identified in Shewanella oneidensis, while phage-borne multidrug efflux pump genes (marA, emrE and mdfA) were detected in E. coli. Collectively, multiple ARG were enriched in clinically relevant bacterial pathogens recovered from a freshwater lake.

Implications/Applications: Continuous surveillance of aquatic ecosystems is crucial to fully assess the burden of AMR in the environment.

Author: Opeyemi Lawal, University of Guelph, Canada

Co-Authors: Bryan, Noah; Soni, Mitra; Chen, Yanhong; Precious, Melinda; Fedynak, Anastasia; Parreira, Valeria; Goodridge, Lawrence

Back to the Program

Session A

Abstract 192

Poster Board number: 49

Cumulative effects of sub-inhibitory erythromycin on soil antibiotic resistance genes

Antibiotic resistance development in environmental community under long-term sub-inhibitory antibiotic contamination remains unclear. Here, we conducted a 5-year field experiment to explore cumulative effects of sub-inhibitory erythromycin on soil antibiotic resistance genes. Erythromycin concentrations in soils were approximately 5 μg/kg and 20 μg/kg in low and high dose experiments, respectively. The soil ARG abundance did not shift significantly during the first and second years, but increased during the third to the fifth year. Macrolide resistance genes were selectively enriched. The abundance and diversity of erythromycin 23S ribosomal RNA methylase gene (erm) gradually increased within five years, and erm(T) showed the most sensitive response to erythromycin contamination. Multivariate statistical analysis showed that effects of erythromycin, bacterial community and MGEs on ARGs became gradually stronger. Culture-dependent bacterial isolation and culture-independent metagenomic assembly were conducted to disentangle the mechanism of erm dissemination. The erm(T) was located on small plasmids consisting of erm(T), rep and mob, while other erm genes were mainly located on transposable insertion structures or chromosome-derived sequences. The erm(T)-carrying small plasmid was the most abundant contig that was detectable since the first year of field experiment, suggesting that the sensitive response of erm(T) to sub-inhibitory erythromycin might be related to its specific genetic context. Small plasmids with core “ARG-rep-mob” segment show features including potential of conjugative transfer, rolling-circle replication, broad host range and low fitness cost, according to research on clinic and animal-derived bacteria. Small plasmids were further identified as MGE type with high dissemination capacity in environmental media, and played a key role in antibiotic resistance development in environmental community. These results suggested that impacts of sub-inhibitory erythromycin on antibiotic resistance of environmental bacterial community were cumulative and gradual, with significant enrichment of small plasmid carrying erm genes. This work was supported by National Natural Science Foundation of China (32141002).

Author: Ziming Han, Research Center for Eco-Environmental Sciences, Chinese Academy ofSciences, China

Co-Authors: Feng, Haodi; Wang, Chen; Liu, Shihai; Dai, Shiting; Zhang, Yu; Yang, Min

Session A

Abstract 203

Poster Board number: 50

Transmission of antibiotic resistance in a model soil detritus food chain under pesticide stress

Background and Aim: Soil is an important reservoir of antibiotic resistance genes (ARGs), but the transmission routes for ARGs between different trophic levels in soil ecosystems are still poorly understood. Pesticides are also widely detected in soil and recent studies have shown that pesticides can contribute to the spread of ARGs in soil. Thus, it is necessary to deeply understand the transmission of ARGs in soil food chain under pesticide stress for assessing their risk to non-target organisms and human beings.

Method: Here, we studied the occurrence of ARGs in soil collembolan (Folsomia candida) under pesticide (Zinc thiazole, ZT) stress, and explored the trophic transfer of these ARGs between the soil collembolan and their predator, predatory mite (Hypoaspis aculeifer) using high-throughput quantitative PCR. Microbial profiles were examined using 16S rDNA sequencing and high-quality sequences were blasted against reference sequences from multiple bacterial pathogen detection database.

Results: Our study revealed that zinc thiazole (ZT) significantly elevated the abundance of ARGs in soil collembolan microbiome. With the increasing of ARGs in collembolans, an increasing of ARGs in predatory mites was observed through trophic transfer. Results showed that mobile genetic elements (MGEs) were serving as crucial mediators in the transmission of ARGs along this food chain. ZT exposure also altered the microbial composition and structure in this model soil food chain. Moreover, shared abundant potential pathogens (such as Brevundimonas diminuta) in both collembolan and predatory mite guts, were found highly correlated to ARGs.

Implications: Our findings provide insights into transmission of ARGs within soil food chain under pesticide stress and emphasize the vital role of MGEs in this process, highlighting an underestimated route of ARG transmission and potential pathogen dispersal in soil ecosystems.”

Author: Zhelun Liu, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; Newcastle University, United Kingdom

Co-Author: Wang, Yi-fei; Zhu, Dong; Quintela-Baluja, Marcos; Graham, David; Zhu, Yong-guan; Qiao, Min

Back to the Program

Session A

Abstract 408

Poster Board number: 52

Xenogenetic Evolutionary of Integrons Promotes the Environmental Pollution of Antibiotic Resistance Genes

Background and Aim: Environmental pollution of antibiotic resistance genes (ARGs) has been a great public concern. Integrons, as mobile genetic elements, with versatile gene acquisition systems facilitate the horizontal gene transfer (HGT) and pollution disseminations of ARGs. However, little is understood about the characteristics of ARGs mediated by integrons, which hampers our monitoring and control of the mobile antimicrobial resistance risks.

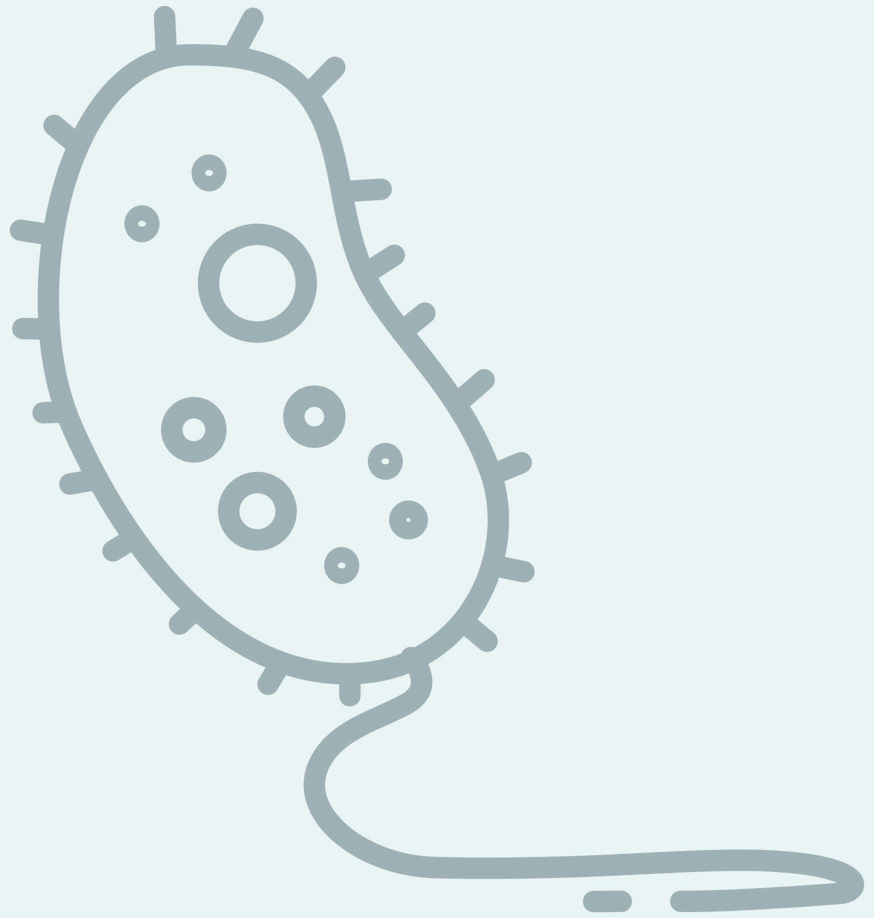
Procedure/Method: To address these issues, we reviewed 3,322 publications and extracted all the individual information, and conducted comprehensive analysis concerning detection methods and pipeline, ARG diversity and evolutionary progress, environmental and geographical distribution, bacterial hosts, gene cassettes arrangements, and based on which to identify ARGs with high risk levels mediated by integrons.

Findings/Results: Integrons exhibit a progressive ability of capturing/accumulating more diverse and multiple xenogenetic ARGs over the past 40 years. Diverse ARGs of 516 subtypes attributed to 12 types were capable of being carried by integrons, with 62 core ARG subtypes prevalent in pollution source, natural and human-related environments. Hosts of ARG-carrying integrons reached 271 bacterial species, most frequently carried by opportunistic pathogens Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae. Moreover, the observed emergence of ARGs together with their multiple arrangements indicated the accumulation of ARGs mediated by integrons, and thus pose increasing HGT risks under modern selective agents.

Implications/Applications: With the concerns of public health, we urgently call for a better monitoring and control of these high-risk ARGs. Our identified Risk Rank I ARGs (aacA7, blaOXA10, catB3, catB8, dfrA5) with high mobility, reviewed key trends and noteworthy advancements, and proposed future directions could be reference and guidance for standard formulation.

Author: Liping Ma, East China Normal University, China

Co-Authors: An, Ran; Qi, Yuting; Zhang, Xu-Xiang



Session A

Abstract 415

Poster Board number: 53

Bacteriophage-associated antibiotic resistance genes are abundant before but not after wastewater treatment

Antimicrobial resistance (AMR) is a major global challenge impacting public health. Wastewater is the major source of antibiotic resistance genes (ARGs) in urban settings, but little information is known about the abundance of ARGs in the viral and free-floating extracellular DNA fractions. These compartments are very relevant to horizontal gene transfer (HGT) events, which are central to AMR evolution and environmental dissemination. Transduction (i.e., HGT mediated by viruses) has received recent attention due to its ARG mobilization and transmission potential. However, due to their sampling approach, most studies only addressed the transduction potential of bacteriophages that are already internalized, possibly dormant, and thus not actively spreading ARGs. This study aimed to elucidate the contribution of the viral and free-floating extracellular DNA fractions in the spread of AMR in wastewater environments. To achieve this, wastewater influent, effluent, and activated sludge were sampled, and qPCR and high-throughput metagenomics were used to assess the abundance and diversity of ARGs carried by bacteria, extracellular DNA, and bacteriophages. These fractions were analyzed separately after a combination of (i) filtration with 0.22-µm membranes (to retrieve bacterial intracellular DNA), (ii) crossflow ultrafiltration followed by PEG precipitation and digestion with DNAse (viral DNA), and (iii) anion-exchange chromatography followed by ethanol precipitation and proteinase K digestion (extracellular DNA). In the influent, ARGs were as abundant in the extracellular DNA and viral DNA fractions (2.0 - 2.5 log gene copies/mL). After wastewater treatment, viral ARGs were hardly detected, corresponding to 1 log (intI1 and sul1) or 2 log (ermB, tetM) removal, whereas extracellular ARGs were still abundant in the effluent. This study evidences the marginal contribution of the viral fraction of wastewater to the overall dissemination of environmental AMR via wastewater. In contrast, surveillance should better integrate extracellular DNA fractions.

Author: Goncalo Macedo, Department of Biotechnology, Delft University of Technology, Netherlands

Co-Authors: Pye, Hannah V.; Wagemans, Jeroen; Lavigne, Rob; Adriaenssens, Evelien M.; van Loosdrecht, Mark CM; Schmitt, Heike; Weissbrodt, David

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 355

Poster Board number: 54

Impact of Wastewater Effluent on Surface Waters Resistomes in a Low-Income Community with Failing Sewage Infrastructure

Background and Aim: BACKGROUND: Wastewater treatment plants (WWTPs) have proven to play an important role in reducing antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in influent sewage prior to release of the effluent to the environment. However, it is important to recognize that WWTPs do not always function as designed. For example, in the U.S., 10% of WWTPs were in violation of the Clean Water Act National Pollutant Discharge Elimination discharge limits. The aim of this study was to assess the influence of the effluent of a WWTP suffering from chronic I&I on the resistome of affected surface waters.

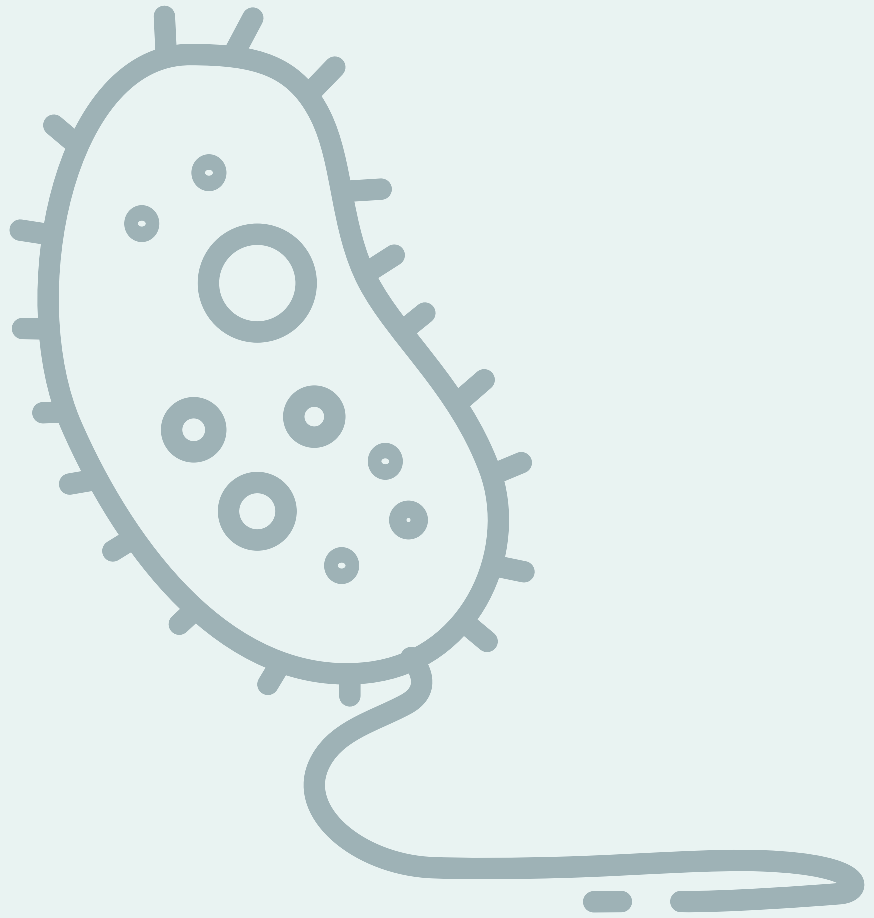
Procedure/Method: The 0.5 MGD WWTP selected for study was located in a low-income rural US community experiencing challenges with aging infrastructure. A 1-year sampling campaign was conducted of WWTP influent, effluent, and surface waters before and after discharge, using 24-hour composite samplers. Culture (cefotaxime-resistant Escherichia coli and carbapenemase-producing Enterobacteriaceae (CPE)), metagenomics, and digital droplet polymerase chain reaction (ddPCR) of intI1 and blaCTXM-1 were performed to obtain multi-dimensional profiles of antibiotic resistance.

Findings/Results: It was found that the WWTP effluent contained an average of 0.36 CFU/100mL and 0.60 CFU of cefotaxime-resistant E. coli and CPE, respectively, and that signals remained detectable downstream. Whole genome sequencing of isolates is currently underway to assess how resistance profiles shift through wastewater treatment and in the riverine environment.

Implications/Applications: This study provides a comprehensive assessment of the impact of a failing WWTP on culture- and molecular-based measures of AMR in affected surface water. The results can help inform AMR-specific risk assessment for surface water exposures."

Author: Gabriel Maldonado Rivera, Virginia Tech, United States

Co-Authors: Pruden, Amy; Vikesland, Peter; Cohen, Alasdair; Markham, Clayton; Darling, Amanda; Davis, Benjamin; Amaral-Torres, Amber; Price, Sarah; Deck, Madeline; Byrne, Thomas



Session A

Abstract 339

Poster Board number: 55

Fate of antibiotic resistance and organic micropollutants in small-scale wetland-based municipal treatment systems

Background and Aim: Human sewage is one of the major sources of antibiotic resistant bacteria, antibiotic resistance genes, and organic micropollutants (OMPs). Wastewater treatment is a crucial barrier to prevent environmental contamination. This study aimed to assess the efficacy of three full-scale constructed wetlands (CWs) that use macrophytes bed as the main process to treat domestic effluents in rural areas.

Procedure/Method: Three CWs operating since 2008 and serving small populations (<200 population equivalent) in Northern Portugal were sampled in the four seasons of 2023-winter (March), spring (May), summer (July), and autumn (October). A total of 36 twenty-four-hour composite samples of influent and effluent, and sediments was processed for enumeration of Escherichia coli and total coliforms on Chromogenic Coliform Agar, DNA extraction (in triplicate), and solid-phase extraction of OMPs. Samples were characterized for culturable enteric bacteria, quantitative PCR of total bacterial inferred from 16S rRNA gene, biomarkers associated with anthropogenic contamination (intI1; uidA; sul1; crAssphage; ermB, ermF, qacEΔ1, tetX, mefC and aph(3´´)-ib)1, 16S rRNA gene metabarcoding, and OMPs quantification by liquid chromatography–high resolution mass spectrometry (LC-HRMS).

Findings/Results: Enterobacterial removal values ranged between 0.94±0.17 and 4.00±0.11 log-units/mL for total coliforms and 0.80±0.11 and 4.75±0.25 log-units/mL for E. coli. Gene removal values ranged between -0.11±0.08 and 3.57±0.09 log-units/mL for crAssphage and mefC, respectively. Pharmaceuticals detected frequently included: pain killers (e.g. acetaminophen), illicit drugs (e.g. cocaine), antihyperlipidemic (e.g. fenofibric-acid), antihypertensives (e.g. irbesartan), or psychoactive drugs (e.g. oxazepam), which were present in all samples (1st and 2nd campaigns) and persisted after treatment. The macrophytes growth stage along the year and climate conditions, such as the temperature, may influence treatment efficacy.

Implications/Applications: The results obtained so far suggest that the CWs can be a good treatment alternative to treat domestic wastewater in small populations without affecting the natural landscape.

1)Teixeira et al. 2023"

Author: Celia Manaia, Universidade Católica Portuguesa, Portugal

Co-Authors: Ribeiro Teixeira, Ana Margarida; Halwatura, Lahiruni M.; Matos, Diana; Coelho, Norberta; Vaz-Moreira, Ivone; Castro, Paula; S. Aga, Diana

Back to the Program

Session A

Abstract 68

Poster Board number: 56

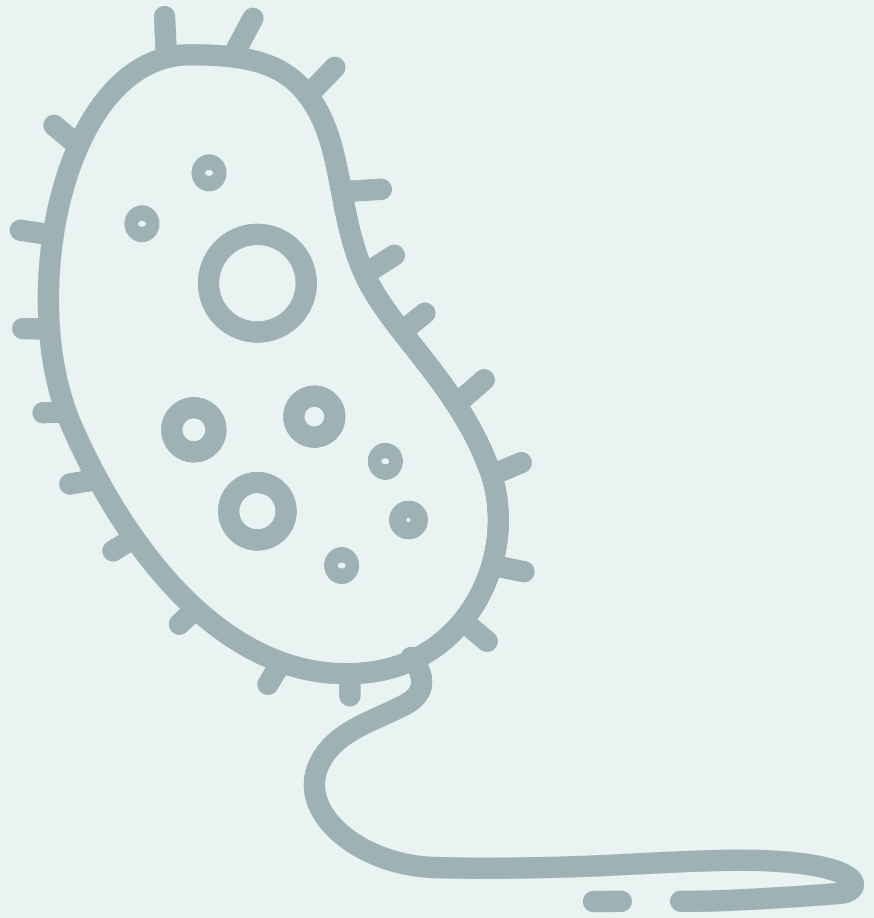
Resistance against last-resort antibiotics in pathogens and novel resistance factors are prevalent in sewage from Norway

Background: The aim was to understand the prevalence and persistent dissemination of antibiotic-resistant Escherichia coli, Klebsiella pneumoniae and antibiotic resistance genes through sewage in Bergen, Norway. Further, we aimed at determining the source of epidemic-causing carbapenem-resistant strains as well as defining the novel resistome of hospital effluent and the receiving sewage treatment plant (STP) in Norway.

Methods: Composite 24h-samples of raw and treated sewage were collected on nine time-points (June 2020-Feb 2022) from five different STPs in Bergen city, Norway. Antibiotic sensitivity was carried out using Sensititre® EUVSEC plates. Whole genome sequencing was performed using Illumina MiSeq and nanopore sequencing. Shotgun metagenomics was carried out using Illumina NovaSeq. Novel genes were predicted using computational models. Results: E. coli (n = 685), K. pneumoniae (n = 607) and cefotaxime-resistant E. coli (n = 567) were analyzed. Carbapenamases like NDM-6 VIM-1 and OXA-244, as well as tigecycline resistance gene tet(X4) were detected in E .coli isolates only from influent and effluent of STPs receiving hospital sewage. All K. pneumoniae strains resistant to colistin had a mutation in the mgrB gene, while tigecycline resistant strains carried a tet(A) variant. A novel conjugative IncHI2 megaplasmid (385 kb) carrying 22 unique ARGs, including 2 copies of VIM-1, was detected in E. coli. Epidemic causing OXA-244-producing E. coli ST-38 strains were identical to clinical strains from Western Europe. A total of 1206 resistance genes were detected in hospital and STP metagenome, with 357 representing novel genes (identity to known genes between 89.8 to 31.1 %), with several genes overlapping across samples. Implications: Our studies shows dissemination of multidrug-resistant pathogens and novel resistance genes into the marine environment through treated sewage in Norway. It demonstrates the important of sewage-based surveillance of AMR in detecting high levels of resistance even in a low prevalence setting like Norway.

Author: Nachiket Marathe, Institute of Marine Research, Norway

Co-Authors: Grevskott, Didrik H; Radisic, Vera; Victor, Manish; Salvà-Serra, Francisco; Moore, Edward RB



Session A

Abstract 328

Poster Board number: 57

The AMR challenge: extended-spectrum beta-lactamase-producing Enterobacteriaceae in two anthropogenically distinct areas in Uganda

Introduction: Aquatic systems are reservoirs of antibiotic-resistant bacteria, and resistance genes, hence fostering the spread of Antimicrobial resistance (AMR) from wastewater treatment plants (WWTPs) and hospital effluent. The PAIRWISE consortium investigated the presence of ESBL-producing Enterobacteriaceae and common determinant genes, blaCTX-M, blaTEM, and blaSHV (previously isolated from clinical settings in Uganda) in WWTPs, hospital effluent, and rivers in two anthropogenically distinct areas in Uganda.

Methods: Effluent samples from hospitals, WWTPs, and rivers were picked from Mbarara and Gulu. The samples were filtered, enriched, and cultured on CHROMagar-ESBL agar for phenotypic characterization and species identification using the VITEK 2 system. DNA was extracted from the positively identified ESBL-producing E. coli and K. pneumoniae isolates using ZymoBiomics Mini-Prep Kit followed by conventional PCR to amplify and compare the resistance genes.

Results: Effluent samples from the hospital, WWTPs, and the River in Mbarara registered presence of either ESBL-producing E. coli or K. pneumoniae isolates. All the samples analysed registered at least one of the three targeted resistance genes. However, effluent from hospital, and WWTP in Gulu had only K. pneumoniae. River samples from the Aswa study area had neither indicators of AMR. 20 E. coli and 10 K. pneumoniae isolates carried the blaCTX-M gene, 22 carried the blaTEM gene, and eight carried the blaSHV gene.

Discussion: More intense anthropogenic activities and associated higher discharge of effluent in River Rwizi catchment, Mbarara contributed to higher incidence of AMR. Similarly, hospital and domestic effluent in Gulu were conducive for the proliferation of AMR. Water samples from Rural River Aswa, Gulu, less impacted by anthropogenic activities had no cases of AMR bacteria.

Conclusion: Occurrence of AMR in surface waters can be attributable to anthropogenic impacts associated with effluent discharge from hospitals, cities and farms. The results contribute towards national and international one-health approach for AMR control.

Author: Charles Masembe, Makerere University, Uganda

Co-Authors: Odong, Robinson; Akoll, Peter; Omara, John; Mayega, Johnson Francis; Katende, George; Ndinawe, Ruth Pamela

Back to the Program

Session A

Abstract 198

Poster Board number: 58

Impacts of chemicals and microbiota from hospitals on the emergence of resistance in sewer systems

Background and Aim: Hospital wastewaters are composed of antibiotic resistant bacteria (ARB) and a cocktail of chemical agents, including antibiotics (ATB) exerting selective pressures. The aim of this study was to clarify the role of ARB and chemicals originating from hospitals in the emergence / dissemination of antimicrobial resistance in mixed municipal wastewater.

Procedure/Method: As part of a field approach, domestic wastewater, hospital wastewater and mixed wastewater (domestic + hospital) were sampled and compared in terms of ATB concentrations and antibiotic resistance. Controlled microcosms were also conducted to assess the contribution of the microbiological versus chemical component of the hospital wastewater exposome. They consisted in mixing domestic wastewaters with either full hospital effluent, its chemical constituents, or its microbiota. They were incubated for 96 hours at 21°C and 100 rpm. Antibiotic resistance was monitored by: (i) assessment of percentage of ARB to ciprofloxacin and cefotaxime, (ii) quantification of antibiotic resistance genes (ARGs) by qPCR, (iii) determination of the sequence variants of ARGs from various antibiotic classes (fluoroquinolones, beta-lactamases, polymyxins, and others) by multiplex amplicon sequencing.

Findings/Results: The proportions of ARB to ciprofloxacin were similar in microcosms that received full hospital effluent or only its chemical component, suggesting that selective pressure rather than community coalescence/immigration was the main factor giving rise to ARB in mixed municipal wastewaters. Some ARG variants were unique to each wastewater type, whilst others were shared. Their profiles in the microcosms suggested that hospital-derived variants could persist and coexist along domestic wastewater variants in some instances (e.g., blaMIR, mdtg). In other cases, hospital variants were not maintained in the final microcosm communities (e.g., blaFOX).

Implications/Applications: This research sheds light on the complex interplay between ARGs and chemical agents form hospitals in municipal wastewater, providing insights into the potential dissemination routes and persistence patterns.

Author: Sarah Naudin, Ecole Nationale Vétérinaire de Toulouse, France

Co-Authors: Imazaki, Pedro Henrique; Dupouy, Véronique; Ferran, Aude A.; Arpaillange, Nathalie; Klimova, Natalia; Hoede, Claire; Ramon-Portugal, Felipe; Gibson, Claire; Barret, Maialen; Frigon, Dominic; Bibbal, Delphine

Session A

Abstract 180

Poster Board number: 59

Metagenomic comparison of wastewater treatment plant effluent and other putative sources of AMR to surface water in urban Mozambique

Background and Aim: Wastewater treatment plants (WWTPs) play a pivotal role in reducing antibiotic resistance pathogens in untreated sewage before it is discharged back into the environment. However, low-income settings often have limited resources to construct and operate a wastewater treatment plant. The aim of this study was to apply metagenomic sequencing towards assessing reduction of antibiotic resistance genes (ARGs) by a small WWTP and comparing effluent with other putative sources of AMR to surface water in an urban community in Mozambique.

Procedure/Method; Weekly sampling was conducted in Maputo, Mozambique, between February 2023 and August 2023, of influent, final effluent, upstream/downstream of discharge into a river adjacent to the WWTP, outfalls into the Maputo Bay, and open drains that lead to the river. Sampling was conducted during wet and dry seasons to assess the seasonal variation in precipitation on the AMR signals.

Findings/Results: Across all sites, beta-lactam ARGs were higher than other drug classes, particularly TEM-1 and TEM-197. These ARGs were significantly higher in the effluent than the influent, suggesting that the WWTP may be operationally challenged. However, human health resistome risk scores derived from MetaCompare 2.0, which takes into consideration co-occurrences of rank 1 ARGs, ESKAPE pathogens and mobile genetic elements, were lower in the effluent than the influent. Relative abundance of total ARGs were much higher than WWTP influent or effluent in the upstream surface water during dry season and in the open drain during the wet season.

Implications/Applications: For communities that invest in WWTPs, it is important to ensure that they are fully functioning and also to evaluate and mitigate other sources of AMR to the watershed. This is especially critical for low-income communities with constrained access to healthcare, where there is greater opportunity for exposure to, and contribution to, environmental reservoirs of AMR.

Author: Loc Nguyen, Virginia Tech, Department of Genetics, Bioinformatics and Computational Biology, United States

Co-Author: Pruden, Amy; Capone, Drew; Brown, Joe; Viegas, Edna; Monterio, Vanessa; Cumbane, Victoria; Cullom, Abraham

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 231

Poster Board number: 60

Dissemination of antimicrobial resistance (AMR) upstream and downstream of a wastewater treatment plant in rural Southwest Virginia, USA.

Background and Aim: The global dissemination of antimicrobials, antibiotic-resistant bacteria (ARB), and antibiotic-resistance genes (ARGs) from human and animal waste into the environment significantly threatens public health. The prevalence of antimicrobial resistance (AMR) determinants in rural surface waters is often attributed to inadequate wastewater treatment, agricultural runoff, and overuse of antibiotics in healthcare. To understand AMR dynamics within rural environments, we investigated ARB and ARG levels in surface water samples collected upstream and downstream of a rural wastewater treatment plant (WWTP) in Southwest Virginia.

Procedure/Method: We evaluated seasonal and location-specific variations in microbial communities and ARGs. Quarterly (summer, fall, winter, spring) grab samples were collected from eight surface water locations between September 2022 and September 2023. DNA was extracted and sequenced via Illumina HiSeq 2500 with 2 × 100 “bp” paired-end reads. Collected sequences were analyzed using Kraken2 for taxonomy classification, Bracken for relative abundance estimation, and Kraken Tools to determine alpha diversity. DIAMOND and DeepARG, both reliant upon the CARD database, were used for ARG classification.

Findings/Results: As expected, we observed changes in both microbial diversity and the make-up of the resistome as a function of season and sampling location. To illustrate location-based differences the collected results will be compared to those obtained in an urban watershed.

Implications/Applications: This work provides insight into AMR dissemination in rural environments.

Author: Idowu Okeshola, Virginia Tech, United States

Co-Authors: Vikesland, Peter

Session A

Abstract 169

Poster Board number: 64

Hospital effluent is as a source for novel antibiotic resistance genes reaching the marine environment in Norway

Background and Aim: Hospital effluents are treated on-site in many countries, but not in Norway. The aim was to understand the role of hospital effluent in the spread of AMR in the environment in Norway.

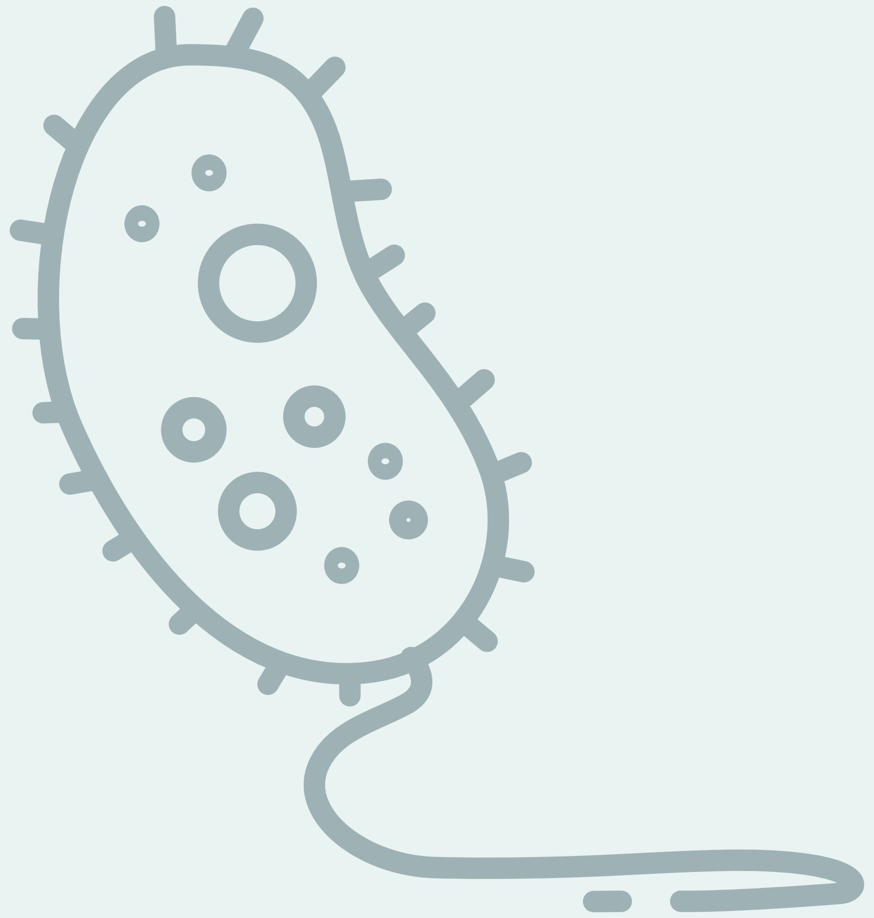
Methods: 24-hour composite samples of the hospital effluent and the influent and treated effluent of the receiving municipal sewage treatment plant (STP) were collected at two sampling time-points (February and April 2023) in Bergen city. Isolation of E. coli and Klebsiella spp. was performed using ECC and SCAI plates with cefotaxime (CTX) (2mg/L), tigecycline (TGC) (0.5 mg/L) or meropenem (MERO) (0.125 mg/L). Antibiotic susceptibility testing was performed using EUVSEC 3 Sensititre® plates. Whole-genome sequencing of E. coli strains (n=23), Klebsiella spp. strains (n=17), and shotgun metagenomics were performed, using Illumina NovaSeq. Known ARGs were identified with USEARCH, while novel ARGs were predicted with fARGene.

Results: All E. coli strains (n=70) obtained from the ECC plates with CTX and MERO were multidrug-resistant (MDR), while 92.3% of the Klebsiella spp. strains (n=55) from the SCAI plates were MDR. The sequenced strains carried multiple clinically important ARGs including different carbapenemases, like blaNDM-5 and blaKPC. Strains from hospital effluent were identical to strains from effluent samples from receiving STP. We obtained approximately 238 Gb of sequence data. In total, >800 ARGs were detected using USEARCH across the samples. Out of 1205 gene sequences assembled using fARGene, 365 were novel ARGs (< 90% amino acid identity), same known and novel ARGs were detected in the hospital effluent and treated effluent from the receiving STP.

Conclusion: Hospital effluent is the source of clinically important ARGs ending up in the marine environment in Norway. On-site treatment of the hospital effluent is needed to prevent the spread of pathogens and ARGs into the marine environment in Norway.

Author: Vera Radisic, Institute of Marine Research, Norway

Co-Authors: Victor, Manish; Grevskott, Didrik; Marathe, Nachiket



Back to the Program

Session A

Abstract 394

Poster Board number: 65

Flash Poster Presentation S2 - Monday, May 27 - 8:30 - 10:15

Sequencing of ESBL E. coli from manure across four countries reveals high prevalence in all regions but distinct resistance patterns.

Background and Aim: Manure fulfills an essential role globally as a renewable source of fertilizer, but antimicrobial resistance (AMR) is a concern following field application. In the JPI ARMIS project, we examined the prevalence of Extended Spectrum Beta-Lactamase (ESBL) producing E. coli from different manure treatment stages in 4 countries and compared genotypic resistance patterns and plasmid associations using whole genome sequencing.

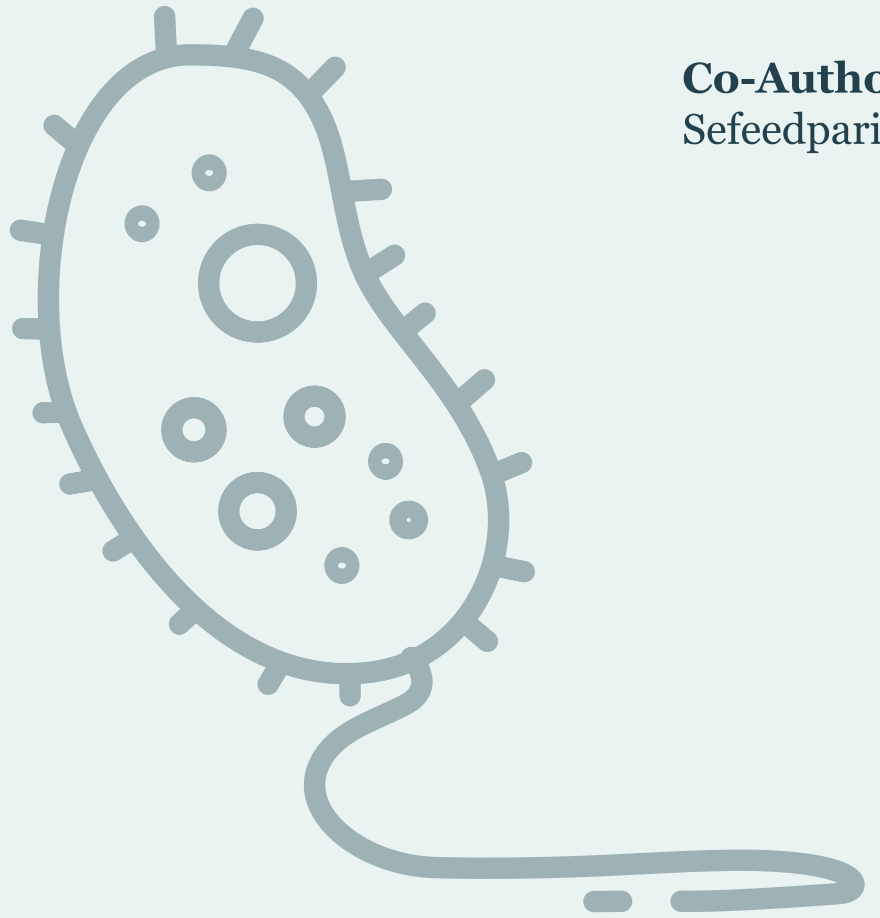
Procedure/Method: ESBL isolates were obtained through both direct plating and enrichment in EC broth + 2 mg/L cefotaxime (CTX), followed by growth on TBX agar + 1 mg/L CTX. Illumina (NovaSeq) sequencing was performed on all isolates as well as Oxford Nanopore (MinION) sequencing on a sub-set of isolates.

Findings/Results: Overall, there was a minimum 3-fold decrease in ESBL detection after manure treatment. We sequenced a subset of 277 ESBL E. coli from Canada (n=62), Germany (n=70), the Netherlands (n=67), and Romania (n=78). The most common lineage identified was ST10 (n=28), which was found in all 4 countries. The average AMR genes per isolate was similar for Romania, Germany and Canada (6.7, 6.9 and 7.6, respectively) and higher for the Netherlands (9.3). The most common ESBL gene identified was blaCTX-M-15 (n=120), which was identified in all 4 countries; however, Canada was unique in having 35% of isolates positive for blaCMY-2. ESBL genes commonly co-occurred with other clinically important resistance genes, on diverse mobile genetic elements, and in a diversity of ST types. The number of AMR genes per isolate was significantly lower for raw manure compared to treated (7.03 genes per isolate vs. 8.41; p=0.006) but only when combining data from all countries.

Implications/Applications: This study demonstrated that diverse manure treatments were effective at reducing the prevalence of ESBL isolates across all countries, though higher resistance per isolate merits further investigation of selection pressures during treatment.”

Author: Nicole Ricker, University of Guelph, Canada

Co-Authors: Chalmers, Gabhan; Glaeser, Stefanie; Popa, Marcela; Scott, Andrew; Topp, Ed; Sefeedpari, Paria; de Roda Husman, Ana Maria; Schmitt, Heike



Session A

Abstract 326

Poster Board number: 66

Natural Transformation is affected by sub-lethal concentration of antibiotics in freshwater bodies conditions.

Background and Aim: Antimicrobial Resistance (AMR) spread is a global health crisis. Freshwater bodies, in which treated wastewater are released, are considered an important route of AMR diffusion in the context of water reuse for irrigation purpose. Despite some evidence suggests that the presence of antibiotics in the environment could influence the evolution of resistance and the spread of ARGs, less is known about how natural transformation is influenced by different antibiotics. The aim of this study was to investigate how different antibiotics at sub-lethal concentrations could affect natural transformation frequency in microcosms in which conditions (i.e., water composition, temperature) typical of freshwater bodies are simulated.

Method: transformation frequency was evaluated in Acinetobacter baylyi BD413, as model strain of natural competent bacteria, measuring its ability to acquire a specific plasmid, in presence of different antibiotics. Sub-lethal concentrations of antibiotics have been firstly chosen determining the minimum inhibitory concentration (MIC) against A. baylyi BD413. Natural transformation has been studied in microcosms with A. baylyi BD413 and the plasmid incubated in presence of antibiotics. Transformants were selected by plating the transformation mix on selective medium,by epifluorescence microscopy and gene specific PCR.

Results: Natural transformation frequency is influenced by the presence of sub-lethal concentration of antibiotics. Natural transformation frequency can differently shift according to the supplemented antibiotic, being favoured by those molecules which resistance is carried on the plasmidic DNA, being unresponsive by those molecules to which the bacteria is resistant or being unfavoured by those molecules which resistance is not carried on the plasmidic DNA.

Implications: This study confirms that the presence of antibiotics influences transformation frequency, underlining how anthropogenic activity could enhance the spread of AMR determinants in environments, such as freshwater bodies, with possible consequences for food safety and human health.

Author: Francesco Riva, University of Milan, Italy

Co-Authors: Mapelli, Francesca; Eckert, Ester M.; Borin, Sara; Crotti, Elena

Back to the Program

Session A

Abstract 375

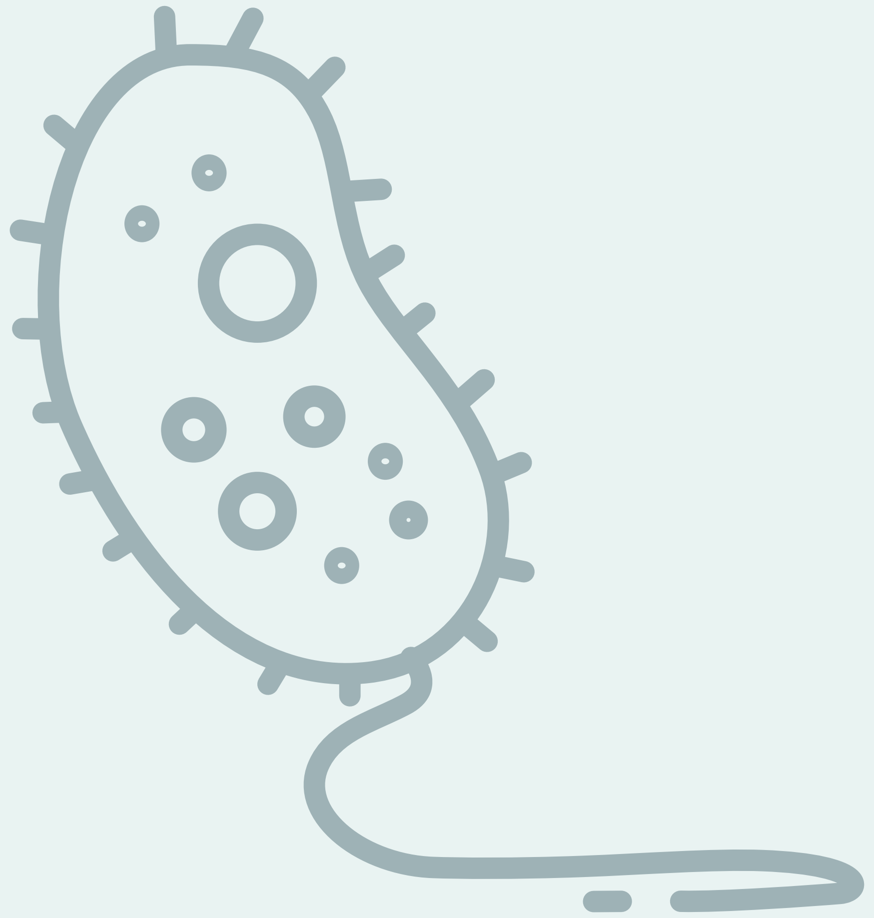
Poster Board number: 67

Seasonal impact of the metropolitan wastewater treatment plant on the resistome and plasmidome of sediments in the Vistula river

The study focuses on the Warsaw wastewater treatment plant (WWTP) "Czajka" and its impact on the resistome and plasmidome of the river sediments in the Vistula River. It aims to understand how urban wastewater influences river ecosystems, particularly in relation to antibiotic resistance genes (ARGs). Effluent samples and river sediments, both upstream and downstream of the WWTP, were collected during the winter and summer of 2023. The research involved bacterial cultivation and genomic DNA isolation from plate wash-outs, raw sediments, and effluents. Illumina sequencing was used for both metagenomic and metaplasmidomic analyses. Bioinformatic work was done using the BV–BRC platform and its Metagenomic Read Mapping tool for ARGs identification. Significant seasonal variation in ARGs was observed, particularly a notable increase in post-discharge sediment samples during summer, contrasting with the stable levels registered in winter. The highest number of ARGs, conferring resistance to at least 18 classes of antibiotics, predominantly beta-lactams and aminoglycosides, was consistently observed in the effluent samples across both seasons. The metagenomic analysis revealed that 225 and 153 different ARGs were identified in the effluents in summer and winter, respectively. Notably, 86 of these ARGs in summer and 17 in winter were also present in downstream sediments, but not upstream. Similar results were noted in the metaplasmidomic data. Both, metagenomic and metaplasmidomic, analyses consistently detected three specific mcr genes (conferring resistance to colistin - a drug of last-resort) in effluent samples across both seasons. In summer, these genes, along with two other mcr variants, were also present in downstream sediments. The study underscores the impact of WWTPs on the dissemination of antimicrobial resistance (AMR) in aquatic ecosystems and indicates ARGs bioaccumulation in river sediments. It suggests that surveillance strategies involving river sediments may be essential for effective AMR mitigation, aligning with the One Health approach.

Author: Anna Rokowska, University of Warsaw, Poland

Co-Authors: Romaniuk, Filip; Dziurzynski, Mikolaj; Dziewit, Lukasz; Swiezewski, Stanislaw



Session A

Abstract 379

Poster Board number: 68

Safer, fit-for-purpose wastewater treatment: focus on antibiotic resistance

Background and Aim: Urban wastewater brings together chemical and biological contaminants, only partially removed during treatment. This work, focused on the variation of antibiotic resistance loads, is integrated in a project that aims to improve urban/industrial wastewater treatment processes and reduce downstream impacts.

Procedure/Method: The study involves three urban wastewater treatment plants (UWWTPs). Samples were collected from influent, decanters, and final effluent and from the receiving river, upstream and downstream of the UWWTP discharge. Enterobacteria were enumerated on chromogenic coliform agar (CCA) and on the same medium supplemented with cefotaxime (CTX) (4mg/L). Isolates were characterized for the antibiotic resistance phenotype and identified by Maldi-TOF. Total DNA extracts were analysed based on selected biomarkers - total (16S rRNA gene), faecal (e.g., uidA) and antibiotic resistance (e.g., intI1, ermF) by quantitative PCR, and bacterial community composition by 16S rRNA gene metabarcoding. The results were analysed and interpreted based on operation conditions and parameters.

Findings/Results: Wastewater treatment led to reductions (from influent to final effluent) of 1.1-1.5 log-units gene copy/volume of total bacteria (based on 16S rRNA gene abundance), of 1.6-1.8 log units of the gene intI1, and of 1.5-3.0 log CFU/volume of total coliforms and Escherichia coli. The impacts of the UWWTP final effluent on the receiving river were negligible, with slight variations of < 0.5 log-units gene copy/volume of the measured genes. CTX resistance prevalence in culturable bacteria ranged 0.8% in coliforms and 4.3% in E. coli. Most of the 164 isolates recovered on CCA-CTX were Enterobacterales with 10.4% presenting multidrug resistance phenotype. The integrated analysis of culturable bacteria, bacterial community composition, biomarkers, physicochemical parameters and operating conditions is proving a valuable approach to adjust operational conditions aiming to increase the removal faecal and antibiotic resistant bacteria, with lower impacts on the receiving environment.

Author: Afonso Ruivo, Universidade Católica Portuguesa, Portugal

Co-Authors: Santos, Isabel; Mena, Cristina; Vaz-Moreira, Ivone; Costa, Cláudio; Faria, Gabriela; Campinas, Margarida; Mesquita, Elsa; Silva, Catarina; Rosa, Maria João; Manaia, Célia M.

Back to the Program

Session A

Abstract 174

Poster Board number: 70

Bacterial dynamics of the plastisphere microbiome exposed to sub-lethal antibiotic pollution

Background and aim: Antibiotics and microplastics are major aquatic pollutants associated to antibiotic resistance selection in the environment. However, little is known about the interaction of these pollutants at environmentally-relevant concentrations and the response of the microbial communities in the plastisphere to antibiotic pollution. The goal of this study was to compare the bacterial dynamics underlying the response to antibiotics at sub-lethal levels in the plastisphere and in surface water.

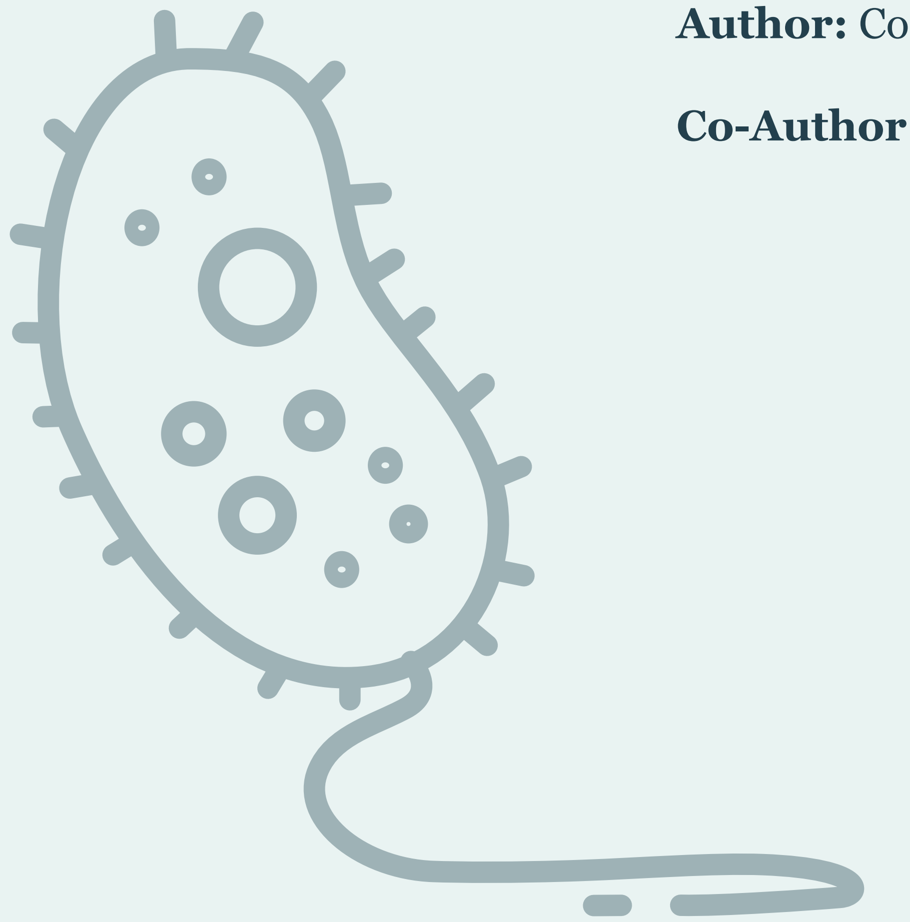
Procedure/Method: River water bacteria were exposed to a combination of antibiotics at sub-lethal concentrations (ciprofloxacin and gentamicin) and /or to polystyrene. The response to these pollutants was evaluated at the community, resistome and mobilome level using a combination of methods (Next Generation Sequencing and qPCR), sequencing targets (16S rRNA gene, pre-clinical and clinical class 1 integron cassettes and metagenomes), technologies (short and long read sequencing) and assembly approaches (non-assembled reads, genome assembly and plasmid assembly).

Findings/Results: The plastisphere showed an increased tolerance to antibiotics and selected different antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs). Several metagenome-assembled genomes (MAGs) derived from the antibiotic-exposed plastisphere contained ARGs, virulence factors and genes involved in plasmid conjugation. These include the opportunistic pathogen Stenotrophomonas maltophilia and other MAGs that belong to genera that have been associated to human infections, such as Achromobacter. The abundance of the integron-associated ciprofloxacin resistance gene aac(6')-Ib-cr increased under ciprofloxacin exposure in both freshwater microbial communities and in the plastisphere, although no significant changes in the mobilome were observed.

Implications/Applications: This study illustrates how the selective nature of the plastisphere influences bacterial response to antibiotics at sub-lethal selective pressure and may help maintain environmental antibiotic resistance. Our research highlights the need to evaluate the impact of aquatic pollutants in environmental microbial communities using complex scenarios with combined stresses."

Author: Concepcion Sanchez-Cid, Université Claude Bernard Lyon 1, France

Co-Author: Joannard, Brune



Session A

Abstract 385

Poster Board number: 71

Effects of Antibiotic use cessation on the soil resistome and microbiome of horse farms: is it enough to curb resistance reservoirs?

Background and Aim: Antimicrobial resistance (AMR), a main global health concern, is linked to the overuse of antimicrobials, especially in animals. Horse farms rely on antimicrobial use (AMU) to control pneumonia caused by Rhodococcus equi, a soil saprophyte. Understanding the interface of AMU and the environment is paramount to combat AMR. Soil microbiomes and resistomes from horse farms were studied prior and after AMU reduction.

Methods: Five horse farms were sampled in 2017 (active AMU) and 2021 (reduced AMU). DNA from soil was shotgun sequenced and used for metagenomic analysis. For all samples, reads were processed, and quality checked. They were used to reconstruct the community and ARG profiles via read mapping using MetaPhLAn4 and ARG-OAP, respectively. The profiles between years were compared using PERMANOVA and Principal Component Analysis.

Results: The soil bacterial communities were dominated by Proteobacteria, Actinobacteria. Interestingly, there was also an abundance of Thaumarchaeota and Nistrospira sp. suggesting that Nitrogen cycling is an important niche in these soils. Microbiomes loosely cluster by year, however no significant difference was observed ($p=0.276$). The number of ARGs decreased from 2017 to 2021 and ARG profiles were significantly different between the two time points ($p = 0.03$). ARG profiles were dominated by multidrug, rifamycin, novobiocin, polymyxin, macrolide and tetracycline resistance, with considerable genetic diversity and sparsity. Analysis of ARGs present in all farms and both time points shows that there is a core of ARGs maintained in the environment despite the reduction/cessation of AMU ($p = 0.059$).

Implications: AMU in horse farms have direct impacts in the ARG profiles of the associated soil environment, with AMU decline leading to lower ARG abundance. However, it does not affect the core ARGs which can act as long-term reservoirs. AMU control should be coupled with other biosafety measures to address AMR spread and selection.

Author: Alinne Santana-Pereira, Auburn University, United States

Co-Authors: Higgins, Courtney; Slovis, Nathan; Gaonkar, Pankaj; Huber, Laura

Back to the Program

Session A

Abstract 66

Poster Board number: 72

Dissemination of antibiotic-resistant bacteria in sewage: is there a correlation between bacterias resistant to different antimicrobials?

Background and Aim: Among the discussions carried out in wastewater based epidemiology, there is the search to reduce the number of samples collected and analyzed, in order to optimize the relationship between the resources used and the representativeness of the results1,2. In general, antimicrobial resistance (AR) studies in WWTP focuses on the monitoring3,4, of microorganisms resistant to a wide range of antibiotics, although the correlation between them is not usually investigated. Thus, although it does not represent causality, correlations between microorganisms resistant to different antimicrobials are capable of guiding, in addition to helping, the proposition of simplified and more assertive monitoring campaigns.

Procedure/Method: In view of the aforementioned objective, the Spearman correlation test was performed, according to data sets determined by Coelho5 and Machado6, over 14 months (2020; 2021; 2022), which contain the concentration of total heterotrophic bacteria (THB) and bacteria resistant to amoxicillin, azithromycin, cephalexin, triazole (sulfamethoxazole + trimethoprim) and meropenem in the raw sewage of the Arrudas and Onça basins by sampling influent from WWTPs located in these sewershed basins.

Findings/Results: Whence, it was possible to observe, in the affected basins, results where meropenem was the only reference antibiotic for antimicrobial resistance that did not show a significant correlation with at least any other bacteria resistant to the other anitibiotics evaluated. The absence of significant correlations, observed among bacteria resistant to all antibiotics other than meropenem (rsmin = 0.34; rsmax = 0.76), can be attributed, from the perspective of the literature, to the exclusive use of this medication in a hospital environment7.

Implications/Applications: The significant correlations observed can guide the selection of azithromycin-resistant organisms (rsmin = 0.50; rsmax = 0.76) for simplified monitoring of the spread of RA, when using resources to maximize the monitored area, while these ARB's were significantly correlated with higher Spearman coefficients.

Author: Juliana Calábria de Araújo, Universidade Federal de Minas Gerais, Brazil

Co-Author: Santos, Henrique

Session A

Abstract 149

Poster Board number: 73

Resistance to critically important antibiotics in meat sold for consumption

Background/aim: Meat sold for consumption by humans and companion animals in the UK is regulated to ensure it falls within range of the bacterial limit deemed safe, however, it is not tested for resistant opportunistic pathogens (e.g. Escherichia coli). Multiple studies show a strong association between feeding dogs a raw meat diet and increased risk of carrying E.coli resistant to critically important antibiotics. It is possible that animals eating raw meat increases the risk of spreading resistant pathogens to others in the household. Here, we investigate 60 samples of meat (beef,chicken,lamb,pork) sold for human consumption (MHC) and 15 samples of chicken raw dog meat (RDM) for resistant (R) E.coli.

Procedure/Methods: Samples were enriched and plated onto amoxicillin,amoxicillin-clavulanate,cefotaxime,ciprofloxacin,spectinomycin and streptomycin to select for R-E.coli. Isolates (max. 10 per plate) were selected and tested against all 6 antibiotics for phenotypic resistance profiles. One resistance phenotype per sample was selected for whole genome sequencing (Illumina) (n=200 R-isolates), determining sequence types and resistance genes. Phylogenetic analysis was carried out using core-genome alignment.

Findings/Results: Highest sample-level positivity rate for R-E.coli in MHC was chicken, 100% of samples were positive for resistance to spectinomycin and streptomycin, and 50% to critically-important fluoroquinolones. Similarly, sample-level positivity in chicken RDM was 87%, 87% and 47%, respectively. Differences were seen in sample-level positivity for cefotaxime; 27% for RDM and 13% for MHC. Colistin resistance gene mcr-10 was found in MHC. The most common STs in RDM were ST10,ST162,ST744. In our previous study on fluoroquinolone resistance in dogs these STs were strongly associated with feeding a raw food diet (phylogenetic analysis will be presented at the conference).

Implications/applications:This study highlights that meat that is not cooked carries multiple R-E.coli, commonly including resistance to critically important antibiotics important for human health. Appropriate hygiene practises after handling dogs fed raw meat is advised.

Author: Jordan Sealey, University of Bristol, United Kingdom

Co-Authors: Avison, Matthew; Astley, Beth; Rollings, Kate

Back to the Program

Session A

Abstract 351

Poster Board number: 74

Flash Poster Presentation: S4 - Monday, May 27 - 11:00-12:30

Evaluation of the implementation of the strategic plan for surveillance and control of antimicrobial resistance 2018-2022 in Senegal.

Background and Aim: Like other countries in the world, Senegal has elaborated a national action plan to combat antimicrobial resistance (NAPAMR 2018-2022) based on a One Health approach, the implementation of which has improved the International Health Regulations' capacities. However, this plan had not yet been evaluated in 2022, making it urgent to analyze the actual level of implementation of the said NAPAMR in Senegal.

Procedure/Method: It was a mixed design study: (i) Quantitative component: cross-sectional, retrospective, and evaluative with a structured closed survey on coverage and implementation indicators; (ii) Qualitative component: phenomenological with a semi-structured open interview guide to identify the determining factors of implementation. Covering the 2018-2022 implementation period (5 years), the study focused on the key sectoral actors in the control of AMR in Senegal. The analysis used Excel2010® and QDAMinerLite1.4.1©2004 software. The approval of the ethics committee was obtained.

Findings/Results: Human, animal, and environmental health were represented respectively at 73.9%, 8.7%, and 4.3%. The regional level was represented at 2.2% too, while sub-regional levels were not. Transdisciplinarity coverage was adequate at 45% and effective at 40%, with an overall achievement of 53.6%. Those specific to the laboratory and hygiene were respectively 70.6% and 69.2%. Implementation was delayed overall by 37.3%, and specifically by 75% for coordination, communication, and research, with 38.1% of activities re-planned. Temporal analysis shows an overall decreasing five-year trend, with better achievements in annual transition (23% in Q4 2020), especially for hygiene, IPC (54%). Finally, the determining factors for implementation were funding, M&E including research, training, integration, and COVID-19 as a blocking factor, hence the usefulness of an M&E framework taking risk mitigation into account.

Implications/Applications: NAPAMR was moderately implemented and needed more funding and M&E for more efficiency and effectiveness.”

Author: Ibrahima Mamby KEITA, Ministry Of Health And Social Action, Senegal

Co-Authors: LEYE, Mamadou Makhtar Mbacké; CISS, Mamadou; DIOP, Khadija Sandembou; WILANE, Mouhamed Bachir; SAMB, Ngone; DIALLO, Amadou Alpha; DIOUF, Coumba; NIANG, Ahmadou; NDIAYE, Adjaratou Diakhou; SOW, Ahmed Iyane; SECK, Ibrahima

Session A

Abstract 263

Poster Board number: 75

Antibiotic Resistance Gene Abundance and Transfer in Wastewater-Irrigated Soils

Background and Aim: Wastewater containing pathogens, antibiotics, and antibiotic resistance determinants raises concerns about the risk of antibiotic resistance genes (ARGs) spreading to the environment, mobilizing from the resistome, and transferring to potentially pathogenic bacteria. ARGs can be disseminated among diverse bacteria via conjugative transfer through mobile genetic elements (MGEs) such as plasmids and integrative conjugative elements.

Procedure /Method: The relative abundance of MGEs and ARGs typically present in Gram-positive bacteria was assessed using TaqMan qPCR on community DNA from three soil types. After eight weeks of incubation in microcosms, these soils were sampled four days and four weeks after irrigation with either untreated or treated wastewater spiked or not with antibiotics and disinfectants. A fluorescence-labelled plasmid system is being developed as a molecular monitoring tool for horizontal gene transfer among Gram-positive bacteria. It will be applied to study the dissemination of ARGs in wastewater-irrigated soils.

Findings/Results: Soils irrigated with spiked wastewater fortified with antibiotics and disinfectants showed a higher relative abundance of plasmids belonging to the pSK1 family, regardless of the type of soil. The relative abundance of pI258 family plasmids as well as dfrD, dfrG, and ermA resistance genes only increased in soils irrigated with unspiked wastewater and slightly decreased when spiked wastewater was applied. A strong positive correlation between the presence of erythromycin and trimethoprim resistance genes (dfrD, dfrG, and ermA), and Inc18-type and pI258-like plasmids was observed.

Implications/Applications: Therefore, our preliminary results suggest that antibiotics and disinfectants can increase the abundance of certain ARGs in wastewater-irrigated soils. The data also provide insight into possible co-selection of ARGs and MGEs from Gram-positive bacteria. Exogenous plasmid capturing via the fluorescently labelled monitoring tool along with 16S rRNA gene amplicon sequencing will enhance our understanding of the role of plasmids and soil type-dependent microbiota on the dissemination of ARGs.

Author: Kornelia Smalla, Berliner Hochschule für Technik, Germany

Co-Authors: Soufi, Leila; Gallego, Sara; werner, katharina; Lüneberg, Kathia; Schakowski, Jana; Quoc, Viet Vu; michaelis, claudia; Heyde, Benjamin; Siemens, Jan; Siebe, Christina; Grohmann, Elisabeth

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 288

Poster Board number: 76

Assessing the reproducibility of whole-microbial community evolution of AMR: unraveling the contributions of vertical and horizontal gene transfer

Background and Aim: Propagation of antimicrobial resistance (AMR) is poorly understood within complex microbial communities. The human gut microbiome contains members of various taxa whose interactions with antibiotics may propel AMR to evolve in a myriad of ways. Understanding how, and how reproducibly, the human gut microbiome evolves under antibiotic selection pressures and other external influences, such as exposure to extracellular AMR genes (eARGs), is critical to furthering our understanding of AMR propagation in realistic microbial communities.

Procedure/Method: Repeat passaging of five replicate samples of a pooled human stool sample was carried out for 14 days with and without exposure to kanamycin and extracellular kanamycin resistance genes (via plasmid pBAV1k-T5-GFP). Over the course of the experiment, samples were characterized via culturing, digital droplet polymerase chain reaction (ddPCR), and 16S rRNA sequencing to assess the reproducibility of whole-microbial community evolution of antibiotic resistance.

Findings/Results: All five replicates of passaged human stool samples evolved high levels of phenotypic resistance to kanamycin. In concordance with repeated passaging, the microbial community became less diverse, shifting to a community dominated by Enterococcus species. The role of the eARG was also determined to be significant; while addition of eARG did not increase levels of phenotypic resistance, it did lead to significant levels of gene uptake (as quantified by ddPCR) and contributed to taxonomic changes in the microbial community.

Implications/Applications: This study improves the current understanding of competing and synergistic processes that lead to evolution of a complex microbiome under antibiotic selection pressures. The approach used herein is applicable to further bench scale studies of other types of clinically- or environmentally-relevant microbial communities (e.g., wastewater treatment plants, soil, vaginal, oral, etc.). This work is also critical to informing patient care or environmental protection efforts as it elucidates potential effects of prescribed antibiotic regimens and environmental eARG exposures.

Author: Lisa Stabryla, University of Illinois at Chicago, United States

Co-Authors: Keenum, Ishi; Chowdhury, Nadratun; Servetas, Stephanie; Dootz, Jennifer; Hunter, Monique; Jackson, Scott

Session A

Abstract 91

Poster Board number: 77

Selective Colonisation of Microplastics by Antimicrobial Resistant and Pathogenic Bacteria

Background and Aim: The independent threats of microplastics and antimicrobials to One Health systems are well documented, yet the combined interactions and role these may have in driving emergence, spread and fixation of antimicrobial resistance (AMR) are not well understood. There is also a lack of evidence to support the potentially unique role of microplastics in enriching for AMR pathogens in microplastic-associated biofilms ('the Plastisphere'). This study aimed to investigate selective colonisation by a complex sewage community on environmentally aged microplastics (polyethylene, polypropylene and polystyrene) in comparison to natural (wood), inert (glass) and planktonic controls. We also investigated whether surface roughness was an important factor in selecting for AMR in the Plastisphere.

Procedure/Method: Selective agar plating was used to explore the diversity and phenotypic AMR profile of culturable bacteria and qPCR was used to determine molecular AMR prevalence (using the intI1 gene). PCR was used to identify Escherichia coli (E. coli) phylo-groups that are known to contain several intestinal pathogens (pathotypes). Following this, polyethylene pellets were mechanically weathered and the selective colonisation of AMR bacteria was compared with virgin counterparts using the same methods as above.

Findings/Results: Opportunity to attach resulted in higher AMR and numbers of pathogenic E. coli, but importantly, the type of substrate mattered when selecting for these traits. Specifically, polystyrene and wood particles significantly enriched AMR, and polyethylene bio-beads (microplastics adopted in sewage treatment) significantly enriched E. coli pathotypes. We also found that surface roughness did not have a significant effect on AMR colonisation.

Implications/Applications: These findings develop our understanding of the mechanisms responsible for enrichment of AMR in the Plastisphere, including bacterial community dynamics, substrate characteristics and environmental parameters. Future work should focus on the role of the Plastisphere in the evolution of AMR and as a vector for AMR between environments and/or trophic levels.

Author: Emily Stevenson. University of Exeter, United Kingdom

Co-Authors: Rushby-Jones, Owen; Cole, Matthew; Lindeque, Penelope; Buckling, Angus; Murray, Aimee

Back to the Program

Session A

Abstract 157

Poster Board number: 79

Flash Poster Presentation: S2 - Monday, May 27 - 8:30-10:15

Spatial and temporal spread of antimicrobial resistance in aquatic environments

Background and Aim The presence of antimicrobial resistance (AMR) spans over various environments including aquatic ecosystems. Despite its widespread nature, understanding environmental factors and community dynamics shaping the dissemination and persistence of resistance genes received very little focus so far. Hence, we aimed to assess the spatial and temporal dynamics of antibiotic resistance genes in a diverse set of aquatic habitats.

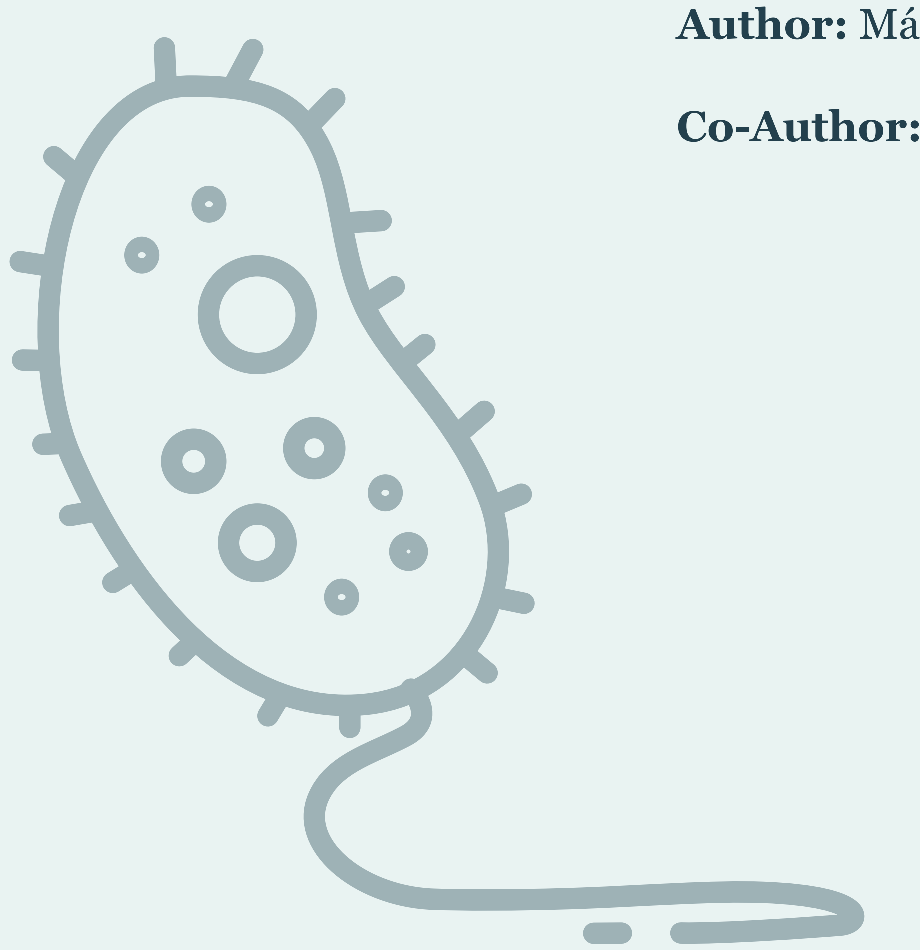
Procedure/Method: Publicly available global metagenomic datasets, covering environments from freshwater lakes/ponds up to various marine environments, were used. To approach spatial mechanisms we estimated horizontal gene transfer processes across different depths of stratified waterbodies, using community-level horizontal gene transfer identification tool (MetaCHIP). We also assessed compositional changes of the resistomes in time and its time-associated correlation with the taxonomic composition of microbial communities and numerous environmental variables by using extended Liquid Association analysis (ELA).

Findings/Results: We quantified resistance gene prevalence in 267 samples from 41 stratified lakes/ponds and 196 marine samples spanning the Atlantic and Pacific Oceans that both cover at least three different depths. Samples for the long-term temporal datasets originated from two marine sites: the North Pacific Subtropical Gyre (n = 68) and the Sargasso Sea (n = 62), capturing a comprehensive representation of environmental variations over two years. The horizontal gene transfer analyses are still in progress at the time of abstract submission.

Implications: Assessing the spatial dynamics of AMR within waterbodies helps us to understand how AMR is regulated by natural stratification of waterbodies and provides basis for modelling its spread when newly introduced genes are released into the environment (i.e., via sewage). From a temporal perspective, changes in microbial community memberships, mediated by environmental conditions, might signal the emergence and/or elevated abundance of antibiotic resistance genes and this could provide relevant knowledge for AMR surveillance programs.”

Author: Máté Vass, Chalmers University of Technology, Sweden

Co-Author: Bengtsson-Palme, Johan



Session A

Abstract 51

Poster Board number: 80

Unearthing the dark side of Glyphosate: boosting antimicrobial resistance and virulence in Klebsiella species.

Background: The shikimic acid metabolic pathway supports the biosynthesis of aromatic amino acids in plants and microorganisms.Glyphosate is a competitive inhibitor of this pathway and a key ingredient in broad-spectrum herbicides such as RoundUp®.Klebsiella pneumoniae is associated with nitrogen fixation in plant roots and has recently been identified as a hitherto unrecognised zoonotic pathogen in the food chain being characterised by its hypervirulence and multidrug resistance in clinical settings.Transient exposure to glyphosate in the environment could lead to changes in susceptibility to antimicrobial compounds in root-associated K. pneumoniae as has been identified in other bacteria and these changes could lead to more severe community acquired infections, that have an impact on public health.

Procedure: In this study,the minimum inhibitory concentration (MIC) of glyphosate was determined by broth microdilution for five Klebsiella isolates.These included the type strain MGH 78578, clinical(K. pneumoniae and K. variicola),environmental (K. variicola), and animal(K. variicola) strains.A sub-inhibitory concentration of glyphosate (0.25 X MIC) was then used to determine whether it exerted any changes on antimicrobial susceptibility against a panel of antibiotics using Sensititre plates.Phenotype microarrays(PM) were also used to further explore any metabolic effects using the same sub-inhibitory selection.

Results: When the Klebsiella species were exposed to glyphosate,a significant increase in resistance to tigecycline and imipenem was recorded in all study isolates.PM results showed that metabolic pathways were affected following exposure to glyphosate, redirecting flux towards the Pentose Phosphate Pathway.Cellular metabolism in glyphosate-exposed strains was also significantly increased at pH 9.5,and this was mirrored by increased crystal violet staining suggestive of an increase in biofilm formation at this pH.

Implications: This study underlines the potential public health risks of environmental glyphosate exposure.It demonstrates that sub-inhibitory glyphosate can extend the antimicrobial resistance phenotype, unmasking resistance to tigecycline and carbapenems, as well as inducing virulence traits such as biofilm formation in Klebsiella species.”

Author: Katie Wall, University College Dublin, Ireland

Co-Authors: Koolman, Leonard; Macori, Guerrino; Fanning, Seamus

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 258

Poster Board number: 81

The overlap of soil and vegetable microbes driving the transfer of antibiotic resistance genes from manure-amended soil to vegetables

Livestock manure, as a major source of antibiotic resistance genes (ARGs), could further transfer ARGs from soil to vegetables when it's used as fertilizer in field and then pose threat to human health. Meanwhile, manure inputs and vegetable planting also affect soil bacterial communities, but these effects on the transmission of ARGs from soil to vegetable is still lacking. Here, lettuce and endive were cultivated in manure-amended soils using pot experiment. The distribution of bacterial community, ARGs and intI1 gene were studied in manure-amended soil and vegetable roots and leaves at harvest. High-throughput sequencing analysis demonstrated that planting vegetables exerted significant effect on soil bacterial communities, which partly explained the decrease of certain ARGs and the intI1 gene in planted soil than in control soil. ARGs in vegetable and soil were interconnected. The bacterial community compositions among root endophyte, leaf endophyte, and phyllosphere were varied by Hierarchical clustering analysis. Higher abundance of shared bacterial taxa was found between root endophytes and soil microbes, which could lead to a relative higher detection frequency of ARGs in root endophyte. Proteobacteria, Actinobacteria, Bacteroidetes and Firmicutes were dominant in the plant endophyte and phyllosphere microbes and had intensive correlations with ARGs. Taken together, our findings provided valuable insights into the role of bacterial community structure in the dissemination of ARGs from manure-amended soil to vegetables.

Author: Fenghua Wang, Hebei Normal University, China

Session A

Abstract 342

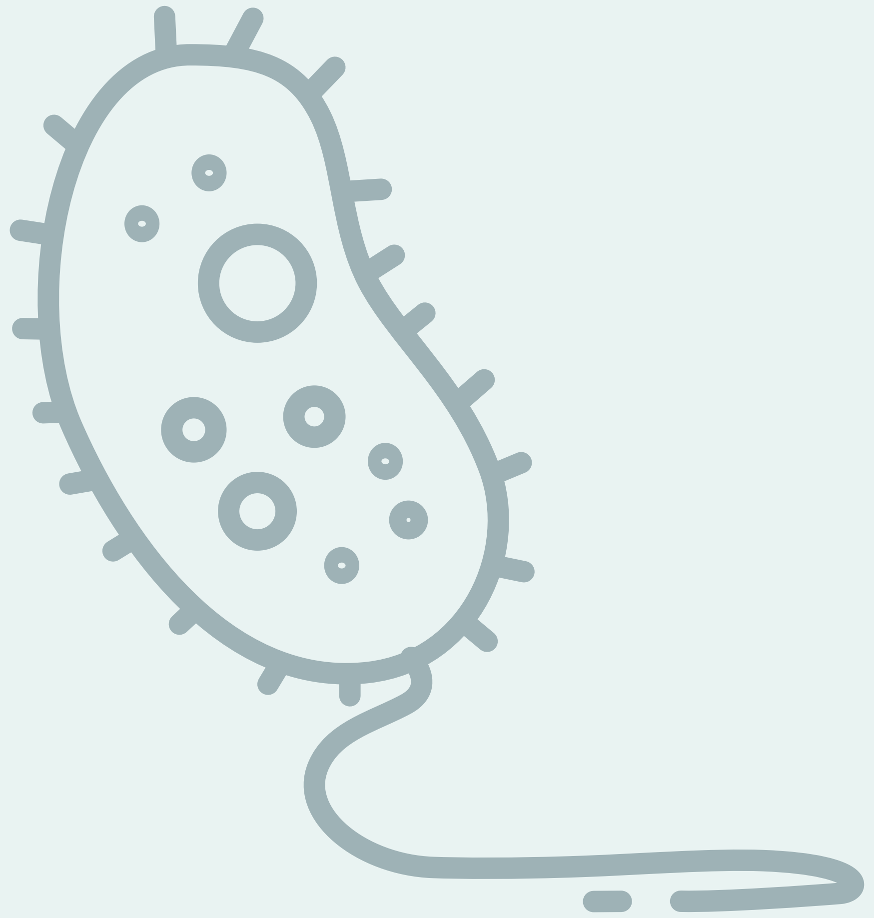
Poster Board number: 82

Molecular Characterization of Extended-Spectrum β-lactamase-producing Escherichia coli isolated from in a River Basin in Yamagata Prefecture, Japan

The distribution and characterization of Extended-spectrum β-lactamase (ESBL)-producing Escherichia coli were investigated at 6 stations from upstream to downstream in Akagawa River, together with another station of Mogamigawa River for comparison, in Yamagata prefecture, Japan. The prevalence of ESBL-producing E. coli among specimens collected from both rivers was 18% (108 of 596 isolates). For the 108 isolates of ESBL-producing E. coli, the possession of the β-lactamase genes (21 genes) was examined using mono-plex PCR analysis. Seventeen of 21 β-lactamase genes were detected in the isolates and the most frequently detected gene was blaCTX-M group-1 possessed by 65 isolates. It is noteworthy that the detected β-lactamase genes included not only blaIMP (9 isolates), an endemic carbapenemase in Japan, but also blaKPC (7 isolates), blaOXA-48 (3 isolates), blaVIM (9 isolates), and blaNDM (5 isolates) which are considered epidemic carbapenemase in foreign countries because of few reports at clinical facilities in Japan. Among seven sampling stations, the highest number (n=15) of β-lactamase genes were detected in the isolates from the station receiving treated wastewater from a municipal wastewater treatment plant. This result implies that healthy people living in the city, as well as patients clinically reported, must harbor ESBL-producing E. coli. All 108 isolates were resistant to ampicillin and cefuroxime and susceptible to tigecycline, and most of the isolates (78 of 108 isolates) were categorized into multi-drug resistance (MDR). Pulse-field gel electrophoresis (PFGE) analysis for these isolates of ESBL-producing E. coli revealed a genetically high divergence among them, suggesting a large variety of its origin in the studied area. To tackle antibiotic-resistant bacteria (ARB), ESBL-producing E. coli has been often surveyed in medical institutions and livestock facilities but the results obtained in the presented study encourage the monitoring of this important ARB in the water environment, which has been rarely reported in Japan.

Author: Toru Watanabe, Yamagata University, Japan

Co-Authors: Nishiyama, Masateru; Mori, Masaya; Yoneda, Ichiro



Back to the Program

Session A

Abstract 208

Poster Board number: 83

Flash Poster Presentation: S2 - Monday, May 27 - 8:30-10:15

Phenomic analyses as means to identify drivers of selection in hospital wastewater

Background and Aim: Wastewaters have been identified as plausible arenas for selection and accelerated evolution of antibiotic resistance. In a recent study, we demonstrated that sterile-filtered, untreated wastewater from a large Swedish hospital strongly and rapidly selected for multi-resistant E coli, using different assays (https://doi.org/10.1016/j.envint.2021.106436). Still, the exact nature of selective agents is unknown. In this study, the aim was to quantify the contribution of different types of resistances on bacterial growth in the presence of hospital wastewater using a novel phenomics approach.

Methods: Untreated municipal wastewater was plated on ECC-Chrome-agar with or without antibiotics, enabling isolation of a large set of E coli. Each strain was subsequently tested for its susceptibility to 23 antibiotics by disc diffusion. To acquire as diverse a collection as possible, combinations of antibiotics as well as replica plating (to find strains that are not co-resistant to specific combinations) were used to complement the collection. As sewage E coli resistant to carbapenems were rare and less diverse, the collection was also complemented with strains from other sources. Individual growth curves were then generated for in total 343 strains in the presence of different dilutions of hospital wastewater using the OmnilogTM platform. More than 60 antibiotics will be analysed by mass-spectrometry in the wastewater.

Preliminary results: A high variability of resistance phenotypes was achieved. Analyses of growth data is just commencing, but the overall pattern is in clear agreement with selection of multi-resistant strains by the wastewater. Machine-learning (e.g. multivariate regression) will be explored as means to identify the relative importance of different resistance phenotypes.

Application: Understanding which resistances are valuable for maintaining growth in hospital wastewater will help identifying individual selective agents, enable risk assessment and direct possible mitigations. The developed phenomics approach could also be applied in other settings to identify drivers of selection.

Author: Noel Waters, Dept. of Infectious Diseases, Institute of Biomedicine, The Sahlgrenska Academy at the University of Gothenburg, Sweden

Co-Authors: Larsson, Joakim; Coertze, Mariska; Ebmeyer, Stefan; Kristiansson, Erik; Flach, Carl-Fredrik; Fick, Jerker

Session A

Abstract 188

Poster Board number: 84

Specialized metabolite from wheat leaves triggers Pseudomonas-antibiotic resistance under host genetic variations

Over the plant evolutionary and/or domesticated time, the plant genetic information was functionally rearranged, gained or lost. Although accumulating evidence suggests a role for plant genotypes of its microbiome, almost nothing is known about how the changes of plant genetic information affect the co-evolved plant microbiome, and its potential influences on antibiotic resistance dispersion. Herein, we experimentally investigated the associations between wheat genetic variation (16 varieties) and their antibiotic resistome in the resident microbiomes. By using amplicon sequencing, high-throughput qPCR, metagenomic analysis and non-target metabolic analysis, we show that host genetic variation remarkably influenced the wheat antibiotic resistome, moreover, specialized genotypes harbor higher levels of ARG abundances in their phyllosphere. Interestingly, we found that the wheat genotypes with high ARG abundances in phyllosphere accompany with an elevated population of Pseudomonas. Metagenomic analysis further revealed that 22 MAGs from Pseudomonas, almost all of which carrying more than two ARGs. Furthermore, we queried the 350 Pseudomonas genome from the global and encompassing a wide range of habitats, to assess the presence of ARGs. Of the all the Pseudomonas strains contains multiple ARGs, VFGs and MGEs on their genome. These findings indicating that the bloom of Pseudomonas in wheat phyllosphere is the main contributor of antibiotic resistance. Moreover, direct links among the upregulated leaf metabolite DIMBOA-Glc, Pseudomonas and phyllosphere ARG enrichment was observed. It was further supported by the microcosm experiment evidenced that DIMBOA-Glc could significantly enhanced the relative abundance of Pseudomonas in soils. Overall, the changes of leaf metabolites resulting from genetic variation throughout the course of evolution may prompt the development of a highly specialized microbial community capable of enriching phyllosphere ARGs, which will advance our understanding on whether plants have evolved to actively recruit these communities, and help to deeply reveal the host genetic variation impacts on the plant resistome.

Author: Qian Xiang, Institute of urban environment Chinese Academy of Sciences, China

Co-Authors: Chen, Qing-Lin; Zhu, Dong; Zhu, Yong-Guan

Session A

Abstract 194

Poster Board number: 86

Comparison of genetic traits between cephalosporin resistant Escherichia coli and Klebsiella pneumoniae from human and environmental sources

Background and Aim: The WHO identifies cephalosporin-resistant Enterobacteriaceae, particularly E. coli and K. pneumoniae, as a significant health threat, necessitating a comprehensive understanding of multidrug-resistant gene spread in microbiota through a “”One Health”” approach. Comparative genomics containing community sewage and hospital isolates were used to develop an epidemiological framework for environmental resistome surveillance, enhancing risk assessment and informing mitigation strategies.

Procedure/Method: This study employs culture-based whole-genome sequencing to examine the epidemiology of cephalosporin-resistant E. coli and K. pneumoniae across various environments, including wastewater from high density residential estates, a foreign workers dormitory and a large scale pet center facility housing several hundred pets. The study includes phylogenetic analysis, intrinsic resistance determination, antimicrobial resistance gene (ARG) distribution, and mobile genetic element (MGE) reconstruction.

Findings/Results:The investigation reveals no significant genetic variations between E. coli and K. pneumoniae from different niches. Both species show intrinsic cephalosporin resistance mechanisms, such as penicillin-binding proteins (PBPs), porins, and efflux pumps. Type-IV secretion systems (T4SS) in all isolates suggest potential conjugative transfer of ARGs. K. pneumoniae isolates predominantly contain MexJK-OprM and AdeABC efflux pumps, whereas E. coli isolates are enriched in MdtEF-TolC. Clinical isolates exhibit intrinsic carbapenem resistance. Differences in the AmpR regulator and AmpC gene expression are noted between E. coli and K. pneumoniae, with environmental E. coli relying on ampC promoter mutations for AmpC gene overexpression. The study identifies the pNDM-HK plasmid as a facilitator of AmpC gene overexpression and conjugative transfer of carbapenemase genes, including OXA-181, KPC-2, and NDM-1, among clinical E. coli and K. pneumoniae. Host predictions for these plasmids include E. coli, K. pneumoniae, and Salmonella enterica.

Implications/Applications: This study concludes that current clinical biohazard treatments are effective but there is a need for enhanced antimicrobial resistance (AMR) surveillance due to the potential horizontal gene transfer among ESKAPE pathogens.

Author: Karina Gin, Department of Civil and Environmental Engineering, National University of Singapore, Singapore

Co-Authors: Yuan, Qiyi; Goh, Shin Giek; Hu, Jiangyong; He, Yiliang;

Session A

Abstract 418

Poster Board number: 87

Antimicrobial burden across a gradient of anthropogenic impact on the Lower Great Lakes

Aquatic environments are of particular concern with respect to the threat of antimicrobial chemical contamination and antimicrobial resistance (AMR) activity, though they and other environmental spaces are often neglected in current AMR surveillance systems that largely concern areas of direct antibiotic administration. We propose a spatiotemporal, multi-ecosystem baseline assessment of the prevalence, activity, transmission and fate of antimicrobial resistance genes (ARGs) across gradients of environmental contamination and anthropogenic impacts in Canada’s priority waterbodies with particular focus on algal bloom communities. The potential effects on the broader food web will be assessed to inform risk assessment for ecosystems from human health perspective and land and water management practices. This project will acquire bioinformatics analysis pipelines from, and contribute data to, the assessment of AMR reservoirs and transmission across the One-Health continuum.

Author: Arthur Zastepa, Environment and Climate Change Canada, Canada

Co-Authors: Broadbent, Jordyn; Crevecoeur, Sophie; Reid, Thomas

Back to the Program

Session A

Abstract 118

Poster Board number: 88

The AMR Multi-Stakeholder Partnership Platform: catalyzing a global movement for action against AMR

The over-use of antimicrobials for human, animal and plant infections and diseases is the main driver of antimicrobial resistance (AMR) all over the world. Poor sanitation and a lack of access to clean water amplify the resistant microbe spread. As a result, AMR is a global health and development threat, involving food safety and food security, economic prosperity, and biodiversity. Because AMR does not recognize any borders, it requires a greater coordination, communication, political leadership, interdisciplinary and multisectoral targeted collaboration in a One Health spirit to deliver on the Global Action Plan (GAP), National Action Plans on AMR (NAPs) and, ultimately, the 2030 Agenda for Sustainable Development. The Political Declaration of the United Nations General Assembly on Antimicrobial Resistance in 2016 mandated the ad hoc inter-agency Coordination Group (IACG) to work on concrete recommendations for tackling AMR. The IACG recommended the establishment of the three global governance structure on AMR along with the One Health Global Leaders' Group on AMR (GLG), the Independent Panel on Evidence for Action against AMR (IPEA) and the AMR Multi-Stakeholder Partnership Platform. The AMR Multi-Stakeholder Partnership Platform is a Quadripartite (FAO, UNEP, WHO, WOAH) collaboration on AMR and One Health which catalyses a global movement for action against antimicrobial resistance (AMR) by fostering cooperation between a diverse range of stakeholders at all levels across the One Health spectrum. On 15-16 November 2023, just before the World AMR Awareness Week, the Inaugural Plenary Assembly of the Platform took place at FAO headquarters. More than 130 member delegates from different countries and stakeholder groups (governments, financial institutions, civil society organizations, academic and research organizations, private sector) meet for the first time, while presenting their proposals for joint Action Groups, discussing Platform's collective governance and priorities for the future, such as the UNGA High-Level Meeting on AMR 2024.

Author: Paola Grenni, National Research Council, Water research Institute, Italy

Co-Authors: Di Cesare, Andrea; Latronico, Francesca; Essack, Sabiha; Motriuc, Nelea

Session A

Abstract 211

Poster Board number: 89

Effects of Ozonation and Post-Treatment Biofiltration on AMR in Swiss Wastewater Treatment Facilities

Background and Aim: Switzerland is the first country to implement a national effort to upgrade a majority of its wastewater treatment facilities (WWTP) with micropollutant removal. Ozonation is one of the most commonly employed treatment strategies, and the disinfection and oxidation action of ozone provide an opportunity to also achieve reduction of AMR contamination of receiving waters. In a pilot study, post-oxidation polishing in biological filters was previously observed to recontaminate ozonated wastewater with AMR. In this study, we quantified the effect of ozonation and biofiltration on AMR levels in four full-scale Swiss WWTPs with ozonation and sand filtration and pilot plants employing granulated activated carbon columns.

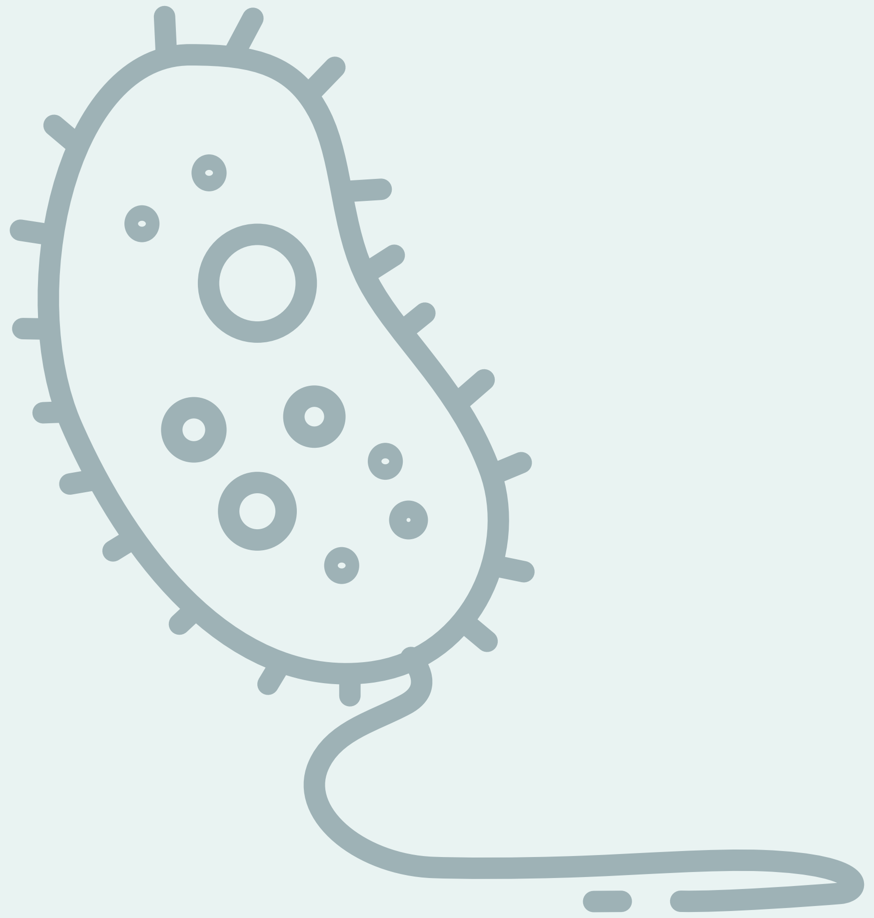
Procedure/Method: Repeated sampling of water before and after ozonation and biofiltration and of biofilter material was performed at six treatment facilities in Switzerland. A combination of cultivation-based approaches and quantitative and metagenomic molecular methods was applied to characterize microbial communities and their resistome.

Findings/Results: Ozonation was found to lead to about one order of magnitude reduction in detectable AMR, confirming earlier results that ozonation at doses used for micropollutant removal cannot completely eliminate viable ARB or ARG from treated wastewater. However, biofiltration in general added to the AMR elimination at most facilities rather than leading to recontamination. No relative enrichment of ARG was observed post biofiltration indicating the lack of AMR selective effects.

Implications/Applications: Current micropollutant treatment strategies that will be applied to most major WWTPs in Switzerland will provide an additional net benefit by significantly reducing AMR release into the environment.

Author: Helmut Bürgmann, Eawag: Swiss Federal Institute of Aquatic Science and Technology, Switzerland

Co-Authors: Yang, Lian; Feng, Ju; Beck, Karin



Back to the Program

Session A

Abstract 5

Poster Board number: 90

Plant-based Natural Products as Potential Phytobiotics

Background: Jaundea pinnata (JP) and Turraea vogelii (TV) leaves have been used in folkloric medicine in Nigeria and other west African countries to treat diseases such as gonorrhoea, syphilis, etc. This informed our choice of testing for their antioxidant and antimicrobial activities against selected clinical bacteria and fungus isolates.

Method: The antioxidant activity of the leaf extracts of Jaundea pinnata and Turraea vogelii in n-Hexane, ethyl acetate, and methanol was determined using Ibok et al. (2023). The minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of crude extracts of JPL n-Hex, JPL Ea, JPL Met, TVL n-Hex, TVL Ea, and TVL Met were determined using the broth micro-dilution method (Taiwo et al., 2023). The microbes were incubated at 37 °C for 24 hours and at 25 °C for 48 hours for bacteria and fungi, respectively. The conventional drugs ciprofloxacin and ketoconazole were used as positive controls, while 1% DMSO was used as a negative control.

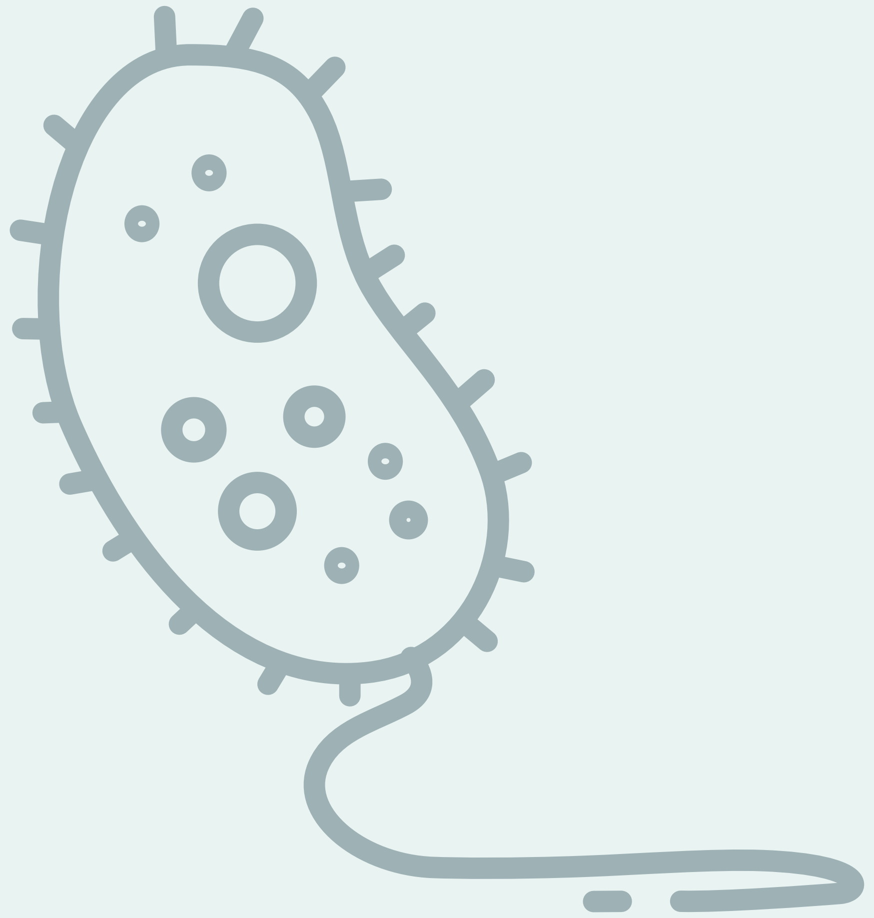
Results: The DPPH scavenging percentage of the plant extracts was concentration-dependent. The IC50s of the extracts were increasing from TVL Met < TVL Ea < JPL Met < JPL Ea < TVL n-Hex < JPL n-Hex, with values of 508.10, 629.07, 707.79, 821.61, 852.05, and 1544.24 (µg/mL), respectively. JPL n-Hex showed a MIC against the test organisms ranging from 0.1953 to 12.5 µg/mL, with MMC ranging from 6.25 to 25 µg/mL. The TVL extracts of n-hexane, ethyl acetate, and methanol exhibited inhibition with MIC ranging from 0.7813–6.25 for both n-Hex and Ea and 0.3906–25 for Met, and MMC ranging from 1.5625–12.5, 3.125–12.5, and 1.5625–25 for n-Hex, Ea, and Met, respectively.

Applications: Medicinal plant extracts could be used in place of standard antimicrobial drugs. The leaf extracts of Jaundea pinnata and Turraea vogelii showed significant activity against bacteria and fungi isolates.

Keywords: Antimicrobials; Jaundea-pinnata; Turraea-vogelii

Author: Ibukun Busari, University of Salford, United Kingdom (UK), United Kingdom

Co-Authors: Aiyelaagbe, Olapeju



Session A

Abstract 238

Poster Board number: 91

Assessing Antagonistic Interactions of Staphylococcus xylosus and Aerococcus urinaeequi Against Bovine Mastitic Staphylococcus aureus

Background and Aim: Bovine mastitis, a primary driver of antibiotic use in the dairy industry, is predominantly caused by Staphylococcus aureus. Previous research has identified Staphylococcus xylosus and Aerococcus urinaeequi as potential probiotics in combating S. aureus mastitis. This study aims to identify probiotic strains of S. xylosus and A. urinaeequi from healthy cows, utilizing three co-culture assays with four S. aureus strains within lineages CC97 and CC151.

Procedure/Method: Assay #1: S. aureus strains, equipped with recombinant plasmids pQS1 and pQS3 carrying the trimethoprim AMR gene, alongside gfpbk or mCherry under the regulation of the S. aureus quorum-sensing (QS) promoter (agrP3), were employed to create a bacterial lawn. Subsequently, S. xylosus and A. urinaeequi strains were streaked onto these bacterial lawns to assess both growth inhibition and quorum quenching (QQ) responses. Assay #2: S. aureus transformants were co-cultured with attackers in TSB over 36 hours, with the fluorescence units of GFP and mCherry measured hourly to quantify QQ or inhibition capacity. Assay #3: Attackers and S. aureus were co-cultured in TSB for 24 hours, followed by measurement of CFU/mL of S. aureus after plating on TSA with trimethoprim.

Findings/Results: A. urinaeequi exhibited notable growth inhibition without QQ, while S. xylosus demonstrated QQ but limited growth inhibition. In Assay#1, 22 out of 28 A. urinaeequi showed growth inhibition, while no A. urinaeequi showed QQ. The majority of A. urinaeequi strains consistently inhibited all tested strains of S. aureus. The A. urinaeequi strains significantly reduced S. aureus CFU/mL. Seven out of 47 S. xylosus exhibited QQ against S. aureus, and only one S. xylosus showed growth inhibition. The S. xylosus strains demonstrated QQ particularly against CC151.

Implications/Applications: S. xylosus and A. urinaeequi from healthy cows could serve as promising probiotics, offering an antibiotic alternative for preventing or treating S. aureus mastitis.”

Author: Zhangbin Cai, McGill University, Canada

Co-Authors: Dagenais, Valérie; Feng, Zhixuan; Ronholm, Jennifer

Back to the Program

Session A

Abstract 411

Poster Board number: 92

ARG removal or intrinsic population selection in anaerobic digestion to limit resistance dissemination? There is the question!

Background/Aim: Municipal wastewater treatment plants receive clinically significant antimicrobial resistance genes (ARGs) from fecal discharges. Thus, optimizing ARG removal is necessary. However, it is not clear if the residual ARGs in the effluent and waste biosolids are from the influent or the intrinsic selection of populations carrying specific ARGs. This was investigated by treatment of waste solids by one-stage mesophilic (35°C) anaerobic digestion (AD), or by two-stage AD configurations, where the first-stage was operated at 35°C under (i) high, (ii) neutral or (iii) low pHs, or (iv) at 55°C, neutral pH, followed by a second stage with mesophilic methanogenic conditions.

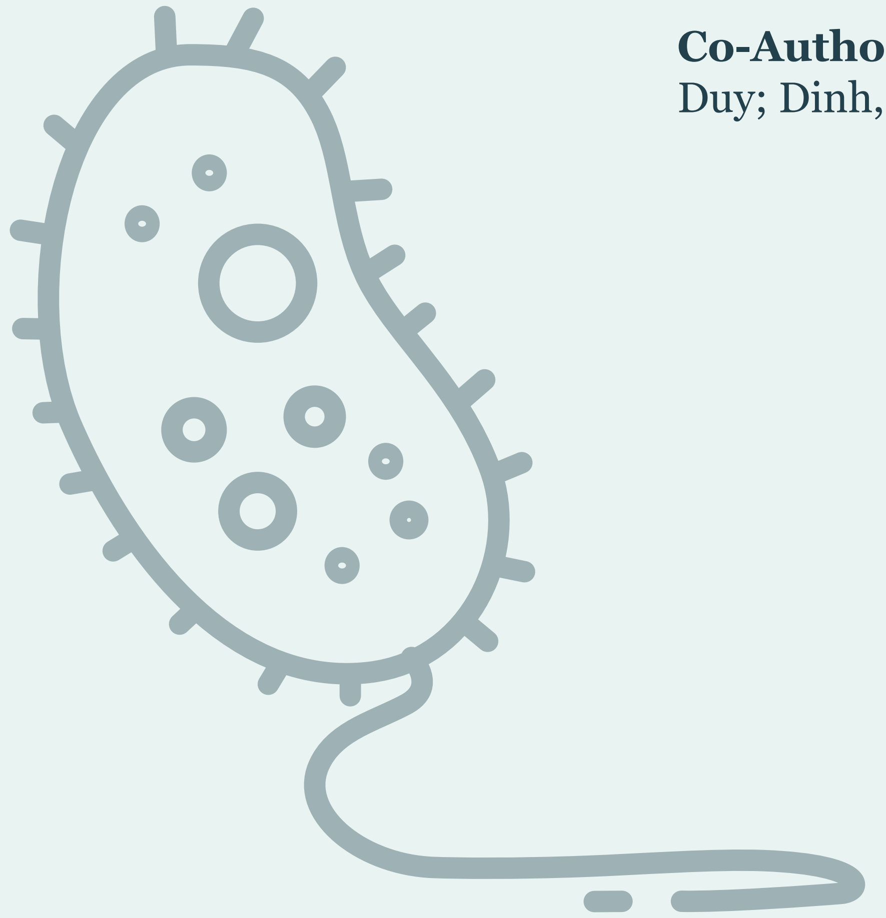
Procedure/Method: Triplicate independent systems were operated for 180 days for all conditions. The reactors were fed separately either primary or secondary sludge because they contain different species and ARGs. The overall SRT was adjusted to 15 days with 3 days for the fermenters. The resistome and community compositions were determined by shotgun metagenomic sequencing.

Findings/Results: Microbial communities differed depending on feed sludge, digestor and fermentation conditions, but solids destructions and methane productions were comparable. Although thermophilic fermentation had the greatest ARG reduction for both sludge feeds, ARG abundances were reduced to similar levels in all conditions, indicating a possible convergent minimum ARG abundance. Consequently, whilst primary sludge-fed systems reduced total ARGs by 83 to 87% and the secondary sludge-fed ones by 26 to 55%, the difference was due to higher levels of ARGs in primary sludge. Finally, the resistomes exhibited clear differences between influent solids, but they were similar in all final digestates.

Implications/Applications: The marginal differences in the levels of ARGs in the final digestates revealed by the side-by-side comparisons suggest minor impacts of AD configurations. Additionally, the similar digestate resistomes independent of feed and AD conditions imply that the natural ARG carriage by intrinsic populations determined ARGs' persistence.

Author: Natalia Klimova, McGill University, Canada

Co-Authors: Caldwell, Catherine; Frigon, Dominic; Jahin, Afia; Kramer, Susanne; Sung, Vo Duy; Dinh, Quoc Tuc; Hassan, Zahra; Sébastien, Sauvé; Walsh, David



Session A

Abstract 308

Poster Board number: 95

Longitudinal Analysis of Antimicrobial Resistance Gene Dynamics in Wastewater across Four Central Canadian Communities

Background and Aim: Antimicrobial resistance (AMR) presents a major global health challenge, yet current monitoring strategies fail to adequately track the dynamics at the community-level. In this study, we explore the dynamics of antimicrobial resistance genes (ARGs) in central Canada with the goal of shaping the design of a national sentinel surveillance program.

Procedure/Method: Weekly influent samples were gathered over nine weeks during the 2022 Winter holiday period from four central Canadian cities, each varying in catchment size and remoteness. For every sample collected, a comprehensive analysis was conducted using a validated quantitative metagenomic approach coupled with an ARG qPCR panel. This analysis involved shotgun and targeted-enriched sequencing.

Findings/Results: Our findings reveal that the levels of ARGs in wastewater were largely stable throughout the nine-week span, indicating a persistent prevalence of AMR during the holiday period. Despite this stability, significant differences were apparent across community types. Remote communities had lower concentrations of ARGs and displayed distinct ARG profiles when compared to urban areas. When adjusted for catchment size, it became evident that per capita, the ARG levels in these remote locations were on par with those found in urban settings.

Implications/Applications: These results have important implications for public health policy and resource allocation. The observed disparity in ARG levels implicates the impact of local elements like population density and isolation on the prevalence of ARGs. Identifying spatial variations in ARGs can guide targeted interventions and resource deployment to mitigate AMR in areas with higher prevalence. Furthermore, this study underscores the urgency of expanding AMR monitoring beyond sentinel locations to include remote communities, as they may also play a significant role in the dissemination of AMR. The observed variations of ARGs across communities in this study will help inform the design of a national sentinel surveillance system for AMR in Canada.

Author: Jade Daigle, Public Health Agency of Canada, Canada

Co-Authors: Dueck, Codey; Wright, Gerry; McArthur, Andrew; Mulvey, Michael; Mangat, Chand; Guitor, Allison

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 272

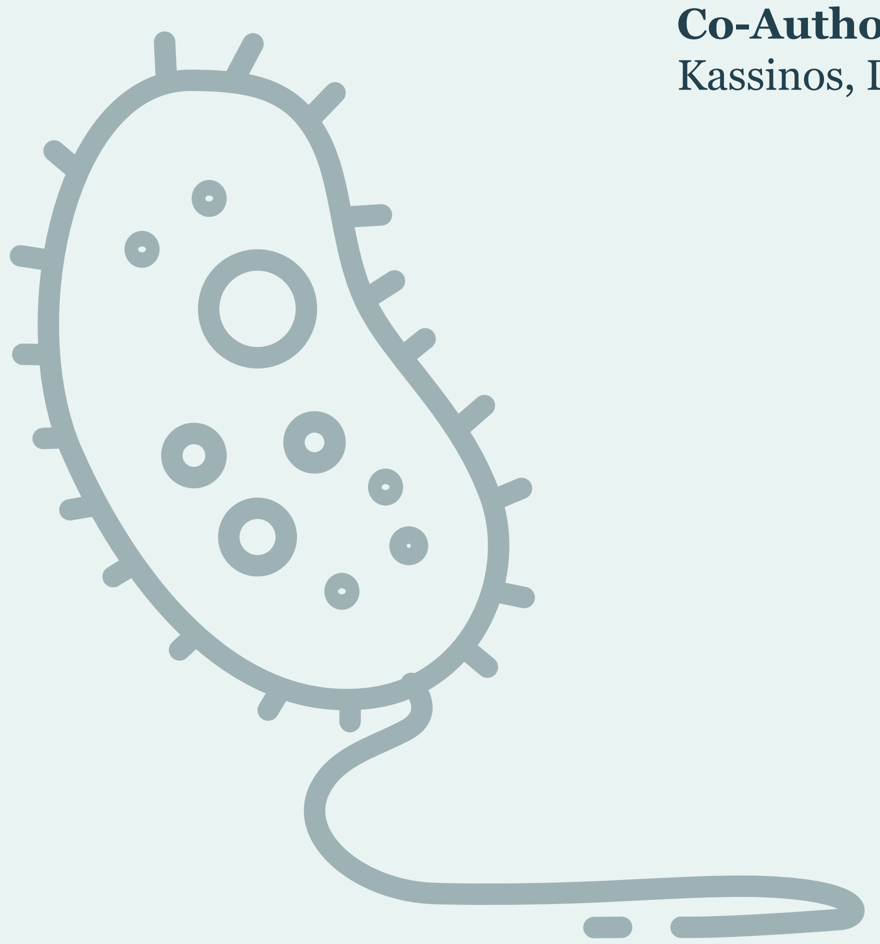
Poster Board number: 96

The influence of in-situ pilot-scale hospital wastewater treatment on antibiotic resistance determinants and total and viable microbial community

The treatment of hospital wastewater using a pilot-scale novel combination of treatment technologies was investigated as to the total and viable bacterial community structure and removal of microbial indicators of health concern including pathogenic bacteria and antibiotic resistance determinants. The pilot-scale system, including anaerobic Membrane Bioreactor (MBR), aerobic Moving Bed BioReactor (MBBR) and UV-C disinfection, was operated for 9 months to treat wastewater from the Larnaca General Hospital in Cyprus. A 2-3 log10 reduction of the ESKAPE pathogens using qPCR analysis, and a 1-log10 reduction of selected genes (16S rRNA, intI1, sul1 and qnrS) were observed at the end of the process. The aerobic MBBR step had the most notable impact on the relative abundance of the selected antibiotic resistance genes (ARGs). 16S rRNA gene amplicon sequencing revealed an overall reduction in the relative abundance of pathogenic bacterial genera in treated effluents. Moreover, analysis of samples treated with propidium monoazide (PMA) for determination of cell viability, has shown a difference of the bacterial communities between live (PMA-treated and amplified) and total (both live/dead, not PMA-treated) cells. Out of the 30 most prevalent putative pathogens in the hospital influent, Desulfovibrio, commonly linked with enhanced biofilm formation and ARG transfer, was highly abundant in influent samples with and without PMA treatment. This finding suggests the general viability of these cells, which may require special treatment focus to prevent their presence in treated effluents. Machine learning bioindicator taxa identification in PMA-free samples for Total Organic Carbon (TOC), Chemical Oxygen Demand (COD) and total nitrogen showed the strongest regressions with the microbial communities having R2 of 81.2%, 75.2%, and 61.2% respectively, and especially with Desulfovibrio and Bacteroides. TOC, NH4 and total suspended solids (TSS) had stronger regressions with bacterial taxa (R2 81.6%, 67.2%, and 66.9%) like Mycobacterium and Reyranella after the PMA treatment.

Author: Iakovos Iakovides, Nireas- International Water Research Centre, Cyprus

Co-Authors: Mina, Theoni; Karaolia, Popi; Vasileiadis, Sotirios; Michael, Costas; Fatta-Kassinos, Despo



Session A

Abstract 281

Poster Board number: 98

Use of conifer needles as passive samplers for antibiotic resistance genes (ARGs) in vicinity of swine

Background: Antibiotic resistance genes (ARGs) related to tetracycline, erythromycin and sulfonamides are frequently found in livestock farms. Swine barns can disperse ARGs in the environment, through ventilation systems, potentially over long distances. Air biomonitoring is usually done by using active air samplers. However, such devices can be expensive and air collection is limited to short periods. Passive air samplers, like conifer needles, could be an alternative to sample the air in the agricultural environments in order to assess the ARG content in the area. This project aims to assess the accumulation of ARGs on conifer needles compared to active air sampling in vicinity of swine barns.

Procedure: Air and conifer needles were collected from 2 control sites located at least 6 km away from any farm of industrial activity. Air samples were taken near the fan exhaust and at 10-30 m and 50-60 m from fan exhaust of two swine barns sites. Conifer needles from swine barns sites were also collected at these distances. Air and conifer needle samples were tested by high-throughput qPCR with a panel of 34 ARGs and 1 mobile genetic element (MGE). This panel includes ARGs for tetracycline (10), erythromycin (5), sulfonamides (2), β-lactams (8), aminoglycosides (3), vancomycin (4), quinolones (2) and the tnpA gene (MGE).

Findings: Concentrations of each type of ARGs (especially tetracycline, erythromycin, sulfonamides and tnpA) from both, air and conifer needle samples, decrease with distance from the swine barns. The lowest concentrations of ARGs are observed at the control sites.

Implications: Conifer needles show a strong potential to be used for the detection of ARGs near swine barns. Further tests are ongoing to verify the consistency of conifer phyllosphere across locations. Results from conifer needle analyses will also be compared to another passive sampler.

Author: Samantha Leclerc, CRIUCPQ - Université Laval, Canada

Co-Authors: George, Paul; Turgeon, Nathalie; Veillette, Marc; Brassard, Patrick; Godbout, Stéphane; Duchaine, Caroline

Monday, May 27 - 16:00-18:00

Back to the Program

Session A
Abstract 378

Poster Board number: 99

Wastewater Chronicles: Comprehensive Analysis of Antibiotic Resistance in Wastewater

Background and aim: Wastewater treatment plants (WWTPs) play a crucial role as reservoirs for microorganisms, encompassing antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs). The primary objective of the CORNELIA project is to enhance our understanding of the environmental impact on the dissemination of antimicrobial resistance (AMR) in a Norwegian context including systematic monitoring of untreated (UWW) and treated wastewater (TWW).

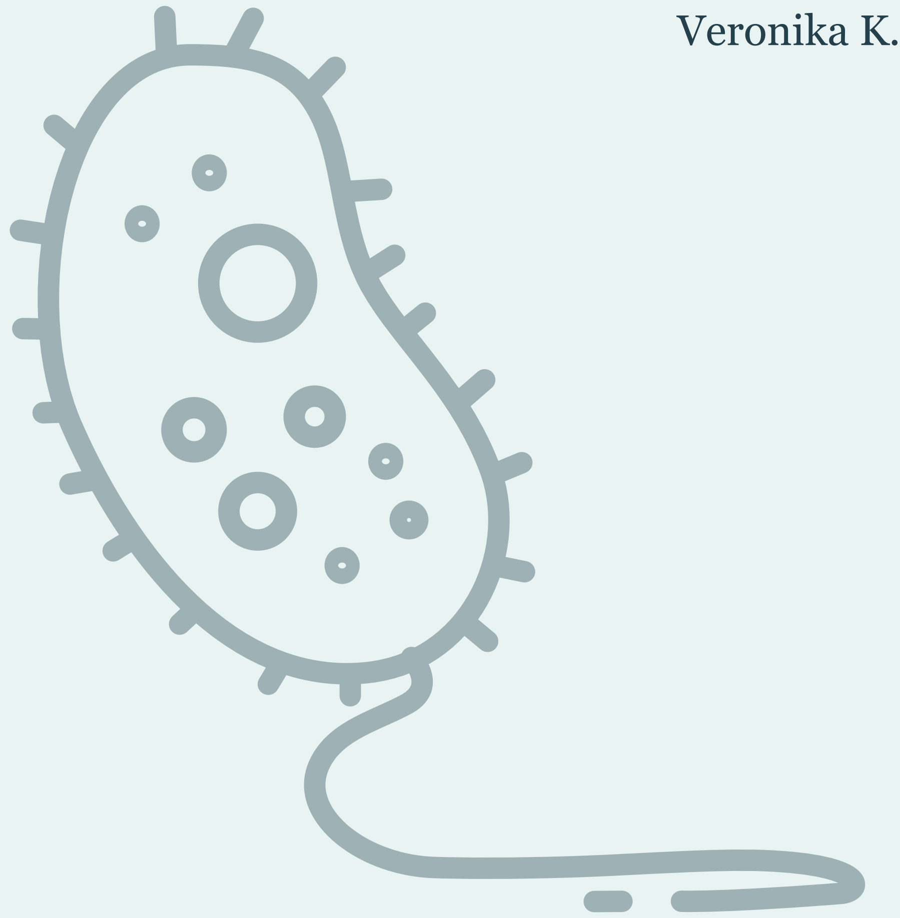
Methods: UWW and TWW samples were collected as 24-hour composite samples over three consecutive days during six sampling events throughout the course of 2022 at VEAS, the largest WWTP in Norway. Samples were processed using three methods: 1) cultivation on selective media for determining the total count of Enterobacteriaceae, extended-spectrum β-lactamase (ESBL-E) and carbapenem-producing Enterobacteriaceae (CPE); 2) a qPCR-based chip to detect 34 clinically relevant ARGs, 3) whole metagenomic sequence analysis of ARGs and bacterial taxa. Information about physical and chemical parameters, as well as the occurrence of various pharmaceuticals in wastewater samples, was furnished for statistical analysis.

Results: The presence of CPE was limited to UWW, whereas ESBL-E were pervasive in all wastewater samples, with abundances reaching 10^5 CFU/ml in UWW and 10^2 CFU/ml in TWW. A total of 21 to 25 different classes of ARGs were identified across the samples, with strA, strB, and tetM exhibiting the highest abundance. Resistome analysis demonstrated a significant reduction in the relative abundance of ARGs during wastewater treatment. Interestingly, the resistome composition appeared to be more diverse in TWW compared to UWW samples. ARGs encoding transferable fluoroquinolone resistance even emerged through the treatment process.

Implications: The study of the CORNELIA project provides key insights for optimal environmental management of wastewater treatment protocols and environmental AMR surveillance methodology, all crucial aspects in addressing the intricate dynamics of ARB and ARGs in a One Health perspective.

Author: Adriana Osinska, Norwegian University of Life Sciences, Norway

Co-Authors: Bargheet, Ahmed; Cuprys, Agnieszka; Fløystad, Ida; Lindstedt, Kenneth; Pettersen, Veronika K.; Sundsfjord, Arnfinn; Wasteson, Yngvild



Session A
Abstract 17

Poster Board number: 101

Flash Poster Presentation: S5 - Monday, May 27 - 13:45-15:30

Compact decentralized advanced treatment methods to combat the release of AMR in wastewater effluents from AMR point-sources.

Background and Aim: Decentralized wastewater treatment at AMR point-sources, such as hospitals, animal husbandries and slaughterhouses, is a potent intervention to reduce AMR spreading into the environment. However, systematic assessments and documentation on the removal effectiveness of local interventions are currently lacking. This information is yet urgently needed by stakeholders and policymakers for economy-driven decision-making. The aim of the work conduced in framework of the JPIAMR HOTMATS project and presented here was to design, demonstrate, and verify compact advanced treatment solutions based on ozone, AOP, UV-C irradiation and membrane filtration (MF) for targeted removal of ARB & ARG and facultative pathogenic bacteria (FPB) in healthcare institution wastewater effluents.

Procedure/Method: Effluents were collected downstream of confined sections of Norwegian hospitals and treated by three pilot-scale systems using O3, UV-C, and MF. The efficiency for removing 384 gene targets focusing on 19 selected ARGs, intl1, 7 ESKAPE taxonomic group indicators, 16S rDNA, was quantified by qPCR.

Findings/Results: We present the first results obtained from pilot-scale tests within the JPIAMR HOTMATS project. Results show that 92% of the 300 gene targets detected in hospital #1 effluent, as well >90% (≥10 log10) of the selected ARGs and taxonomic group indicators were removed by using 20-40 g O3/ m3. The reduction potential for hospital #2 effluents was comparatively lower (75% max.). UV-irradiation of ozonated water increased gene target removal at lower but not at higher (67 g O3/m3) ozone concentrations. Despite of the significant acute inactivation effect some bacteria were able to reactivate after 5 days. For 9 of the 12 micropollutants listed in the proposed EU urban WWT directive 80% removal was achieved using ozone (67 g O3/m3) and UV.

Implications/Applications: The results imply that decentralized wastewater treatment using multibarrier advanced methods can be very suitable to effectively reduce AMR in wastewater from point-sources.

Author: Carsten Ulrich Schwermer, Norwegian institute for water research (NIVA), Norway

Co-Authors: Ingebretsen, Trond; Olafssøn, Halfdan; Xiaoyu, Cong; Schwartz, Thomas

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 432

Poster Board number: 106

Development of pulsed blue light technologies for bacterial biofilm disruption

Background and Aim: Bacterial biofilms protectively shield bacteria, thereby making antimicrobial agents less effective in suppressing bacterial growth. Also challenging is the fact that many bacterial species have become resistant to commonly available antibiotics. As part of an effort to address this problem, we pioneered experiments which showed that 405 - 470 nm blue light suppressed the growth of methicillin-resistant Staphylococcus aureus (MRSA). In recent studies, we showed that pulsed blue light (PBL) is more potent in suppressing bacterial growth than continuous-wave blue light. The potency of PBL makes it a viable antimicrobial for suppressing bacteria growth in biofilms. Consequently, we studied the efficacy of pulsed 450 nm light in suppressing the growth of MRSA and P. acnes biofilms.

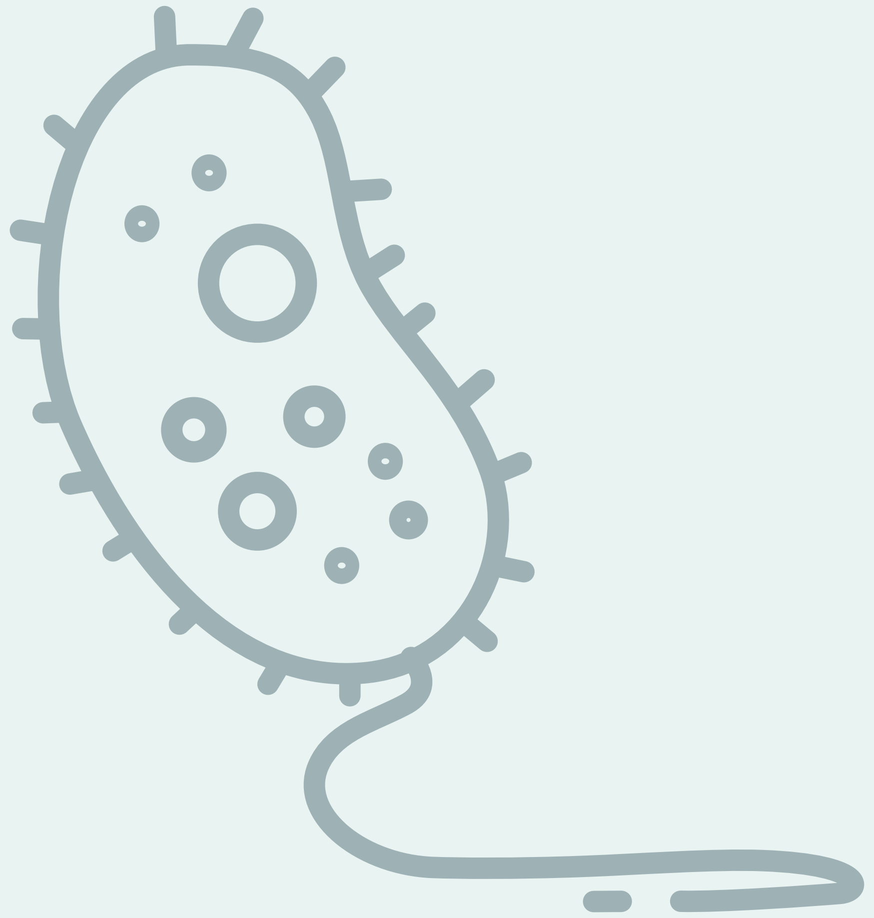
Method: MRSA USA-300 and P. acnes purchased from American Type Culture Collection, were irradiated with 7.6 J/cm2 PBL three times a day, using 3 mW/cm2 irradiance, and in P. acnes planktonic and biofilm cultures irradiated with 5 J/cm2 thrice daily for 3 days, using 2 mW/cm2 irradiance. Plates were incubated at 37°C after irradiation for 24 hours and colonies enumerated.

Results: The results showed 100% bacterial suppression in both planktonic cultures of RSA and P. acnes. However, a similar 100% suppression was not attained in MRSA or P. acnes biofilms irradiated thrice daily for 3 days at various fluences; but LIVE/DEAD assay showed a degree of bacterial suppression, with more live cells in controls than irradiated biofilms, and more dead cells in irradiated than control biofilms. In addition, while control biofilms had intact biofilm networks, irradiated biofilms had disrupted biofilm. The higher the dose, the more bacterial suppression and biofilm disruption.

Implications: Further modifications of the treatment protocol to achieve 100% bacterial suppression in biofilms is in progress. These findings have significant implications in the ongoing effort to mitigate antimicrobial resistance.

Author: Violet Vakunse Bumah, Department of Chemistry and Physics, University of Tennessee at Martin, Martin, TN, USA, United States

Co-Authors: Chukuka S. Enwemeka



Session A

Abstract 187

Poster Board number: 108

Thermophilic ananerobic digestion reduced the abundance but increased the horizontal gene transfer and risk level of antibiotic resistance genes

Background and Aim: Here, three continuous stirred tank reactors were operated for one and half a year (480 days), and the operational parameters covering the addtives, SRT, temperature and TS were concerned to investigate the response mechanisms of ARGs in AD system. The antibiotic resistome were figure out at the reads level, and the phage and ICEs carrying ARGs were separately clarified at the contigs level, while the interaction of virus and bacteria was determined at the metagenome assemble genomes (MAGs) level.

Method: Metagenomics along with bioinformatics analysis are used for the analysis of changes of antibiotic resistome at the reads, contigs and MAGs level. The qPCR of typical ARGs was conducted to elucidate the results from metagenomics. Metatranscriptome was also conducted to clarify the role of horizontal gene transfer of ARGs by mobile genetic elements (MGEs) at the RNA level.

Results: Thermophilic AD increased largely the relative abundance of high-risk ARGs, although it could reduce the relative abundance of total ARGs. The proportion of high-risk ARGs only accounted for 0.89%±0.24% at the thermophilic condition, but it increased by more than five times to 4.16%±0.78% at the thermophilic condition. The conjugative_mobile ARGs accounted for the most as for the antibiotic resistome in AD, that is, 69.2% on average, while the inter_mobile_ARCs accounted for 12.0%. Thermophilic condition significantly increased the conjugative_mobile ARGs from 61.4%±2.8% to 75.8%±3.6% ($p<0.01$), The phage transduction was clearly found for the MLS_mefA, MLS_Isa, lnuC, erm(47) and cfrC, although some of the phages was assigned as lytic lifestyle.

Implications: Thermophilic anaerobic digestion is thought to be effective to reduce ARGs in the swine manure, but the HGT and high-risk ARGs caused by thermophilic condition are neglected. This study provides a new perspective when considering the migration of ARGs for the anaerobic digestion technology.

Author: Junya Zhang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, China

Co-Authors: Lu, Tiedong; Wei, Yuansong; Richnow, Hans-Hermann

Back to the Program

Session A

Abstract 89

Poster Board number: 109

Dirty habits: potential for spread of antibiotic-resistance by black-headed gulls from waste-water treatment plants

Background and Aim: The growth of antibiotic resistance is a major concern for people, yet most initiatives to date that aim to address this issue have ignored the role of nature. Anthropogenic habitats such as wastewater treatment plants (WWTPs) and landfills can be hotspots for antimicrobial resistant bacteria (ARB) and they attract wildlife such as black-headed gulls (Chroicocephalus ridibundus) that may be vectors for ARB dissemination. Previous research indicates how gulls shed antibiotic resistant E. coli in their faeces after being infected. The aim of this study is to investigate the expected spatial patterns of ARB dispersal by black-headed gulls in Southern Iberia.

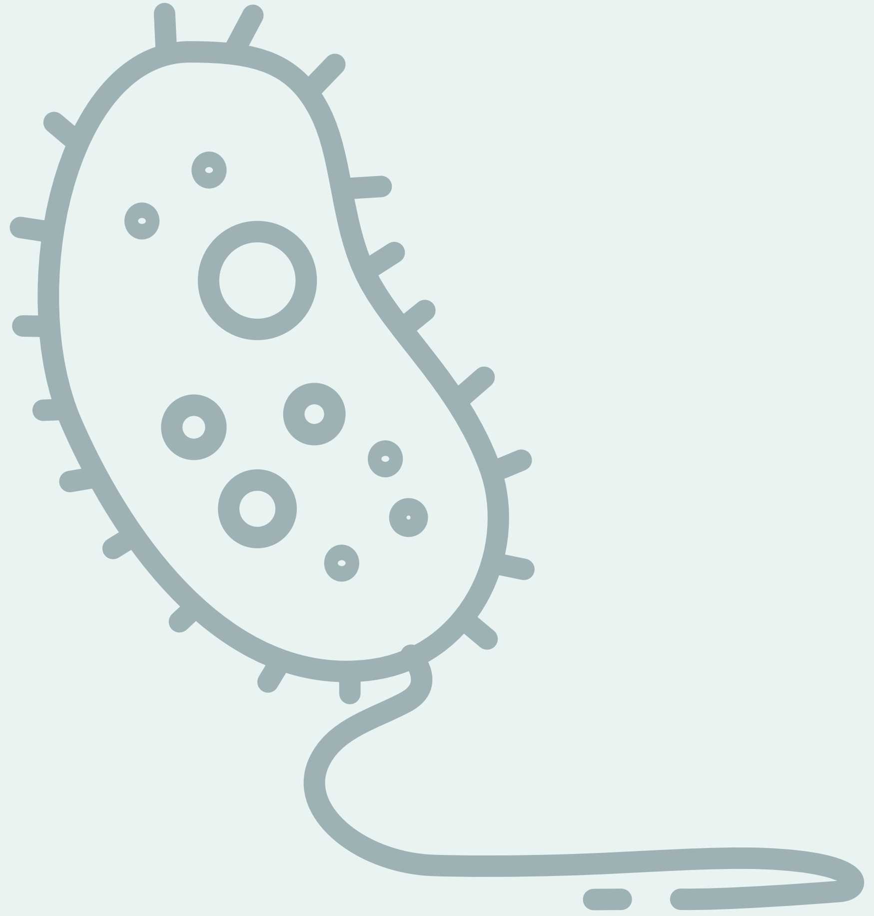
Procedure/Method: As a case study, we used GPS tracking data and land use layers, combined with an ARB shedding curve, to determine likely sources and dispersal distances of ARB in south-west Spain and southern Portugal.

Findings/Results: Tracking of 39 Black-headed gulls for up to eight months revealed that they used WWTPs (21 different ones and 684 visits) more frequently than landfills (three different ones and 21 visits), and that both may be important sources for ARB dissemination into areas of diverse land-use. Estimated dispersal distances of ARB from WWTPs or landfills averaged 25 km for areas of higher risk of spatial dissemination, but reached up to 500 km. Solar saltworks and natural waterbodies were the habitats most exposed to ARB dissemination, followed by agriculture, sports facilities (especially golf courses), and recreation beaches. There was important variability between individual gulls in their habitat specialization and which WWTPs they visited.

Implications/Applications: Studying the spatial movement patterns of gulls can help to pinpoint sensitive locations where they can pick up ARB (e.g. WWTPs and landfills), and where pathogen transmission to people is most likely (e.g. urban parks), facilitating the design of risk reduction measures.

Author: Víctor Martín Vélez, Marine Sciences Institue-CSIC, Spain

Co-Authors: Navarro, Joan; Vazquez, Manuel; Navarro-Ramos, Maria Jose; Bonnedahl, Jonas; van Toor, Marielle; Bustamante, Javier; Green, Andy



Session A

Abstract 401

Poster Board number: 112

Determination of Chemical Pollutants as Drivers for Antimicrobial Resistance in an Agricultural Watershed

Background/Aim: Antimicrobial resistance (AMR), driven by microorganisms' drug exposure and exacerbated by excessive human and animal medicine usage, can be compounded by the influence of pesticides and halogenated compounds in the environment. This study aims to quantify veterinary antimicrobials (VAs), antibiotic-resistant genes (ARGs), pesticides, total adsorbable organic halogens (AOX), pharmaceuticals, and personal-care-products (PPCPs) in an agricultural watershed.

Procedure/Method: In Central Iowa's Black Hawk-Lake watershed, mainly devoted to animal farming, we targeted common VA classes using solid-phase extraction and liquid chromatography-tandem-mass spectrometry. Suspect screening via an existing-database, leveraging liquid chromatography-high-resolution mass spectrometry was performed. The activated-carbon sorbent captured AOX in water samples, and subsequent combustion-ion-chromatography analysis provided overall concentrations of organo-fluorine and organo-chlorine without specific identification. Additionally, 32 genes and mobile genetic elements (MGEs) were screened from a subset of water samples and 8 (16S rRNA, tetT, tetM, tet33, strB, inti3, ermB, and erm35) were quantified on a Biomark Fluorescent machine 192x24 IFC at Iowa State University.

Findings/Results: Targeted analysis detected VAs, primarily monensin (0.5-32 ppb) and erythromycin (0.4-23 ppb), alongside various PPCPs and pesticides from suspect screening. AOX levels were notably high, with total organo-fluorine at 7.9 ppm and total organo-chlorine at 32.5 ppm. The ARGs and inti3 gene measured in the study had similar distributions among the different monitoring sites. Of the total samples and gene measurements, 34.1% were measured below the LOD, 46.9% were measured between the LOQ and LOD, and 18.9% were measured above the LOQ. The two most frequently detected genes were the ARG tet33 and the MGE inti3. These two genes accounted for 46.6% and 48.5% of the total genes measured above the LOQ.

Implications/Applications: The co-occurrence of these components in the watershed points to the importance of considering the presence of other chemical pollutants as selective pressure in the development and spread of AMR.

Author: Damalka Balasuriya, University at Buffalo, United States

Co-Authors: Ahmed, Shoaib; Neher, Timothy; Wijayahena, Mindula; Long, Leigh; Wallace, Joshua; Howe, Adina; Soupir, Michelle; Aga, Diana

Back to the Program

Session A

Abstract 237

Poster Board number: 113

Antimicrobial-resistance genes in bioaerosols from Livestock operations

Background and Aim: Confined livestock operations are known to produce bioaerosols and emit them via their ventilation system. These operations are thought to be contributors to antimicrobial resistance gene development. The objective of this work is to determine the role of bioaerosols in the emission of antibiotic-resistant genes from livestock operations. The specific objectives were to identify ARGs in bioaerosols from livestock operations, evaluate the abundance of ARGs, and evaluate the dispersion of ARGs, from Saskatchewan livestock operations.

Procedure/Method: Two swine and poultry barns were visited twice in summer and twice in winter to determine the presence and dispersion of ARGs. Samples were collected at the exhaust (n=11), at 10m (n=8), 100m (n=8), and 1km (n=8) from the exhaust, and on the opposite side of the building exhaust (n=8). The samples were attained via a high-volume SASS 3100 air sampler and Q-track real-time monitoring. Similarly, five swine gestation barns utilizing stall housing and five utilizing group housing were visited once in winter collecting samples at the exhaust by triplicate (n=30). DNA was extracted from the SASS filters for 16s analysis, and ARGs were evaluated using Takara SmartChip high-throughput Real-Time.

Findings/Results: As the distance from the exhaust increased, the number of total bacteria and particulate matter decreased. The bacteria and ARG diversity changed with distance and livestock housing type.

Implications/Applications: These results enhance our understanding of the role of bioaerosols in AMR and potential sources of ARGs.

Author: Alejandra Castillo Toro, University Of Saskatchewan, Canada

Co-Authors: Kirychuk, Shelley; Duchaine, Caroline; Thompson, Brooke; Nolting, Charly; Lemieux, Joanie; Veillette, Marc; Turgeon, Nathalie

---

Session A

Abstract 240

Poster Board number: 114

Antibiotic Resistance fate: dead, survive or thrive in the microbial jungle depending on shaping effects of associated microbiota in aquaponics

Background and Aim: Since they are saving resources, recirculating aquaculture systems (RAS) are perceived as a viable strategy. They serve the dual purposes of (i) limiting water consumption and (ii) reducing the artificialization of soils. Aquaponics, the most prevalent system, combines aquaculture and hydroponic vegetable production. This system entails a closed-loop water system and continuity between two main microbial “bioreactors,” the plant and fish tanks, with respectively associated rhizosphere and gut microbiota. These two microbial ecosystems are powerful bioreactors capable of shaping microbial community structures by mimicking macroscale environmental interactions through selection, competition, and genetic invasion. This work aimed to clarify the respective role of these two bioreactors when antibiotic-resistant bacteria enter the aquaponics water loop.

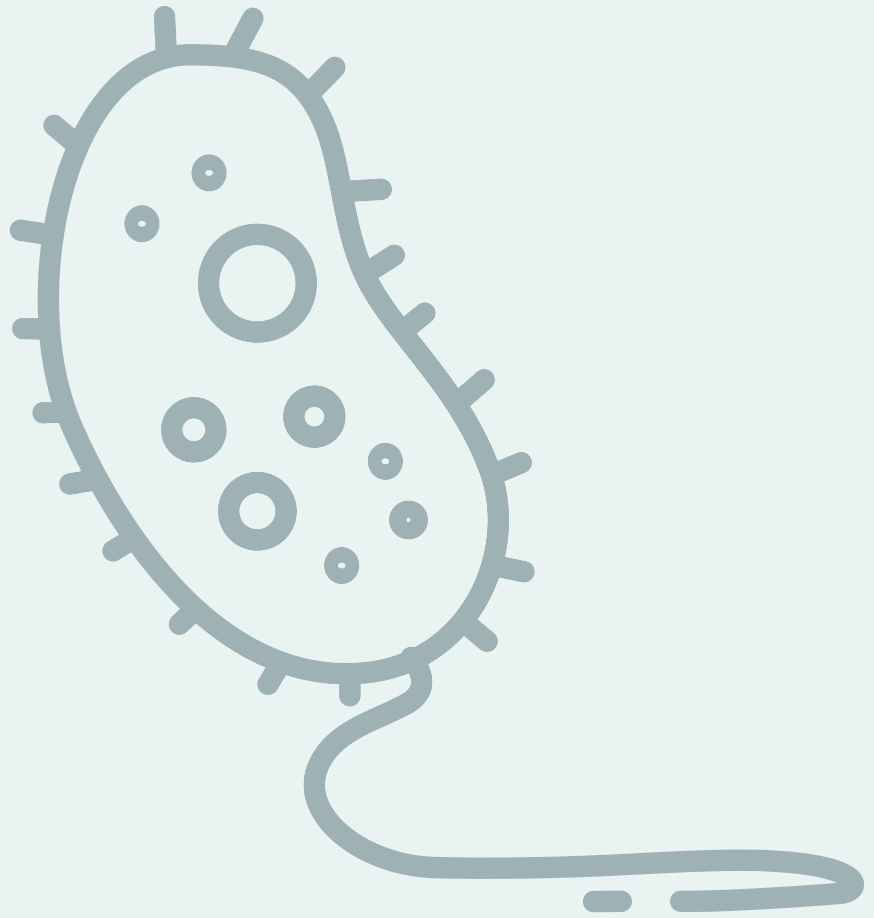
Methods: The fate of antibiotic-resistant genes (ARGs) was investigated on a real-scale farm that offered the opportunity to study the effect of fish species on this dynamic. The ARG composition of four identical independent aquaponic units, differing only by the fish reared, was assessed using a high-throughput qPCR (HT-qPCR) approach on 384 ARGs and mobile genetic elements. Concomitantly, the microbial community structure and biomass were measured through 16S rRNA gene metabarcoding and 16S qPCR.

Results: The results indicated that while fish presence constitutes an enhancement factor for ARG, rhizosphere sphere seems to act as a mitigation factor on the relative abundance of ARGs. Procrustean analysis revealed that microbial community composition was also a main determinant of ARGs’ composition.

Application: Evidence-based “One health” approaches could provide new opportunities for bridging research and new agricultural practices, and enhancing the knowledge on a global scale to slow down antimicrobial resistance

Author: Frédérique CHANGEY, LCPME - UMR 7564 CNRS - UL, France

Co-Authors: Migel, Juliette; Mathieu, Laurence; Merlin, Christophe



Back to the Program

Session A
Abstract 108

Poster Board number: 115

Application of “under the radar” approaches to identify anthropogenically derived pathogen and AMR indicators in produce and soil

Background and Aim: Intensifying global water and fertilizer shortages have facilitated “circular economy”-based agricultural approaches like wastewater reuse and manure amendment. However, these anthropogenic sources may contain pathogens and antibiotic resistance genes (ARGs) that can be transmitted to humans through ingested produce. Our lab previously determined that antibiotic resistant bacteria and associated ARGs dissipate in crops and soil irrigated with treated wastewater. However, we recently found that short-term inoculation of treated wastewater-irrigated soil in nutrient-rich medium facilitates proliferation of bacterial pathogen and ARG indicators.

Procedure/Method: Lettuce root (rhizosphere) and leaf (phylosphere) samples irrigated with freshwater/treated wastewater and amended with/without chicken manure, were inoculated in brain heart infusion broth under oxygen-rich and depleted conditions. Inoculated samples were analyzed using cultivation-based and molecular (16S rRNA amplicon sequencing, HT-qPCR, shotgun metagenomics) methods, to identify resistant bacteria and ARGs. Additionally, we applied mating platforms to assess horizontal gene transfer potential of resistant isolates.

Findings/Results: Inoculation of rhizosphere and phylosphere samples revealed a variety of copiotrophic (fast growing) bacteria that were not detected in direct analyses. Interestingly, while inoculation of freshwater irrigated samples primarily resulted in enrichment of Bacillus spp. and intrinsic ARGs, inoculation of manure-amended and treated wastewater-irrigated crops facilitated growth of bacterial pathogen indicators (i.e. ESBL-producing E. coli and Klebsiella spp., Acinetobacter spp. and Aeronomas spp.), and ARGs frequently associated with these indicators. Furthermore, the “”anthropogenically impacted”” inoculated samples contained bacteria capable of horizontally transferring ARG-harboring plasmids to sensitive hosts, whereas inoculated samples from the freshwater irrigated crops did not.

Implications/Applications: The described inoculation platform revealed the presence of bacterial pathogen and associated ARG indicators in anthropogenically impacted crops that were not distinguishable in direct analyses. We are currently focusing on exploiting this novel platform in quantitative microbial risk assessment models that underline epidemiological risks associated with treated wastewater irrigation and manure amendment.

Author: Chagai Davidovich, Institute of Soil, Water and Environmental Sciences, Volcani Institute, Israel

Co-Authors: Cytryn, Eddie; Erokhina, Kseniia; Blum, Shlomo

Session A
Abstract 392

Poster Board number: 116

Can anaerobic digestion reduce the prevalence of antimicrobial resistance genes in UK poultry litter?

Livestock manure is known to be a significant hotspot for antibiotic residues, antibiotic resistant bacteria (ARB), antimicrobial resistance genes (ARGs) and co-selecting agents. This is often spread onto fields as fertiliser, allowing antimicrobial resistance (AMR) to enter the environment, potentially contaminating the food chain and water bodies. This study investigated the prevalence of antibiotic resistant Enterobacterales in the litter of three contrasting poultry farming systems in the UK, and laboratory-scale mesophilic anaerobic digestion (AD) of litter was carried out. High-throughput qPCR was used to determine the fate of 96 ARGs/MGEs during AD, and 16S amplicon sequencing was used to determine changes in the bacterial community structure during AD. Multi-drug resistant Enterobacterales were found in all three farming system litters. Up to 63 ARGs were detected in the three farming systems, and a total of 37 ARGs across all litters were significantly impacted by AD. Other genes, such as IntI1, were unaffected by the AD process. The prevalence of ARGs detected in this study highlights the need for poultry litter sanitation before land-application. This study also provides a comprehensive insight into ARG behaviour during mesophilic anaerobic digestion of different poultry litters and demonstrates that AD can reduce the relative abundance of some ARGs, but not all.

Author: Sarah Guest, University of Nottingham, United Kingdom

Back to the Program

Session A

Abstract 370

Poster Board number: 118

Effects of antimicrobial use on minimum inhibitory concentrations in Salmonella, Campylobacter and E. coli isolates from poultry.

Background and aim: Antimicrobial resistance (AMR) is a major concern for “One Health”. Injudicious antimicrobial use (AMU) contributes to the development and spread of multidrug-resistant bacteria. The antimicrobial consumption in food animals is projected to increase over time. Thus, it is important to monitor AMU and their impact on the development of AMR. The objectives of this study were i) to determine temporal trends in minimum inhibitory concentrations (MIC) in Salmonella spp., Campylobacter spp., and E. coli for commonly used antimicrobials in poultry; ii) to assess the impact of AMU and other farm management factors on MIC shifts over time; and (iii) to forecast AMR.

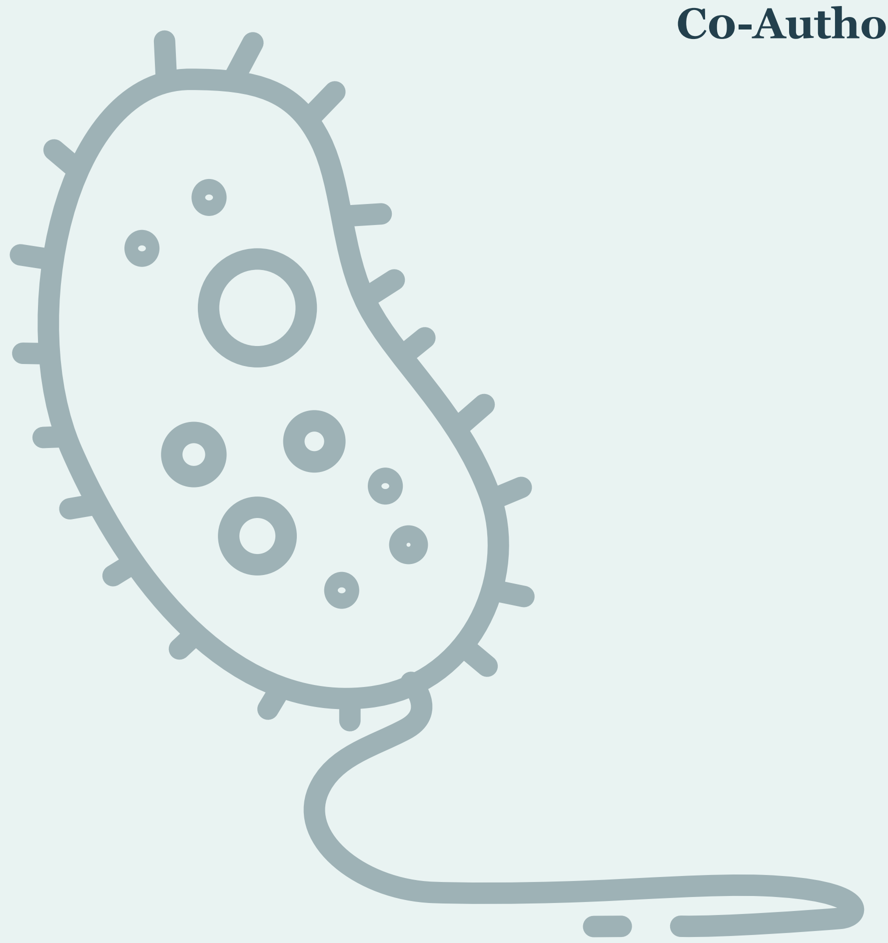
Methods: Farm-level data were collected by the Canadian Integrated Program for Antimicrobial Resistance Surveillance. AMU data were collected via questionnaires and AMR was determined using broth microdilution applying the CLSI breakpoint guidelines. Temporal trends in AMU and MIC data from 2013 to 2023 were explored to determine the effect of AMU and management factors on the shift in MIC over time.

Results: AMU in poultry has decreased over the last decade, except for bacitracin which has increased since 2018. MIC have either decreased or maintained constant for most antimicrobial classes, except for tetracycline and nalidixic acid MIC which have increased over time in Salmonella spp. and Campylobacter spp., respectively. Tetracycline AMR is projected to increase in the next decade, whereas AMR to streptomycin and sulfamethoxazole are projected to decrease.

Implications: Continued monitoring of AMU and AMR data is necessary to develop AMR mitigation strategies and to evaluate the effect of measures to reduce AMU on animal welfare, food production, and food safety. The use of MIC values as an alternative to the use of interpretation criteria is important for detecting subtle changes in AMR levels for faster implementation of mitigation strategies.

Author: Laura Huber, Auburn University, College of Veterinary Medicine, United States

Co-Authors: Agunos, Agnes; Gaonkar, Pankaj; Gow, Sheryl; Carson, Carolee; Molinari, Roberto



Session A

Abstract 387

Poster Board number: 121

Composting reduces the risks of resistome in beef cattle manure at the transcriptional level

Background and Aim. Proper treatment of manure before land application is essential to mitigate the spread of antibiotic resistance in the environment. Stockpiling and composting are two commonly used methods for manure treatment. However, the effectiveness of composting on reducing antibiotic resistance in manure has been debated. Transcriptomic evidence is needed to determine if composting is more effective than conventional stockpiling in mitigating the risk of resistome in livestock manure. The objective of this study is to compare composting and stockpiling for their effectiveness in reducing the risk of antibiotic resistance in beef cattle manure.

Procedure/Method. Samples collected from the center and the surface of full-size manure stockpiling and composting piles were subject to metagenomic and metatranscriptomic analyses.

Findings/Results. While the distinctions in resistome between stockpiled and composted manure were not evident at the DNA level, the advantages of composting over stockpiling were evident at the transcriptomic level in terms of the abundance of antibiotic resistance genes (ARGs), the number of ARG subtypes, and the prevalence of high-risk ARGs (i.e., mobile ARGs associated with zoonotic pathogens). DNA and transcript contigs show that the pathogen hosts of high-risk ARGs included Escherichia coli O157:H7 and O25b:H4, Klebsiella pneumoniae, and Salmonella enterica. Although the average daily temperatures for the entire composting pile exceeded 55°C throughout the field study, more ARG and ARG transcripts were removed at the center of the composting pile than at the surface.

Implications/Applications. Our results demonstrate that composting reduced more high-risk resistance genes at the transcriptomic level in cattle manure than conventional stockpiling. This finding not only underscores the effectiveness of composting in reducing antibiotic resistance in manure but also highlights the importance of employing metatranscriptomic analyses alongside metagenomic analyses.

Author: Xu Li, University of Nebraska-Lincoln, United States

Co-Authors: Sun, Yuepeng; Staley, Zach; Woodbury, Bryan; Riethoven, Jean-Jack

Back to the Program

Session A

Abstract 305

Poster Board number: 122

Flash Poster PresentationS19 - Thursday, May 30 - 13:45-15:30

The co-occurrence and potential transfer risks of antibiotic resistomes between non-intensive aquaculture environment and human

Background and Aim: Aquaculture is an important food source worldwide. Using antibiotics in intensive large-scale farm has resulted in the resistance development. Non-intensive aquaculture is another aquatic feeding model that is conducive to ecological and environmental protection. However, transmission of resistomes in non-intensive aquaculture has not been well characterized. Moreover, the influence of aquaculture resistomes on human health needs to be further uncovered.

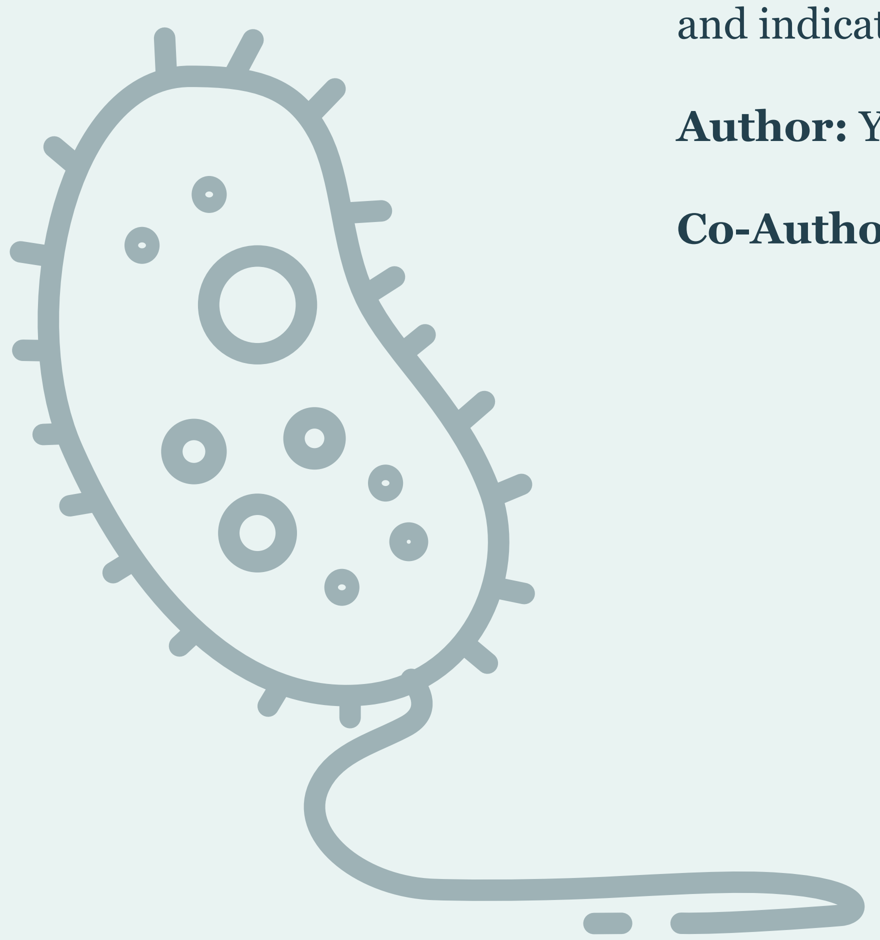
Procedure/Method: To analyze the sharing of resistomes between aquaculture system and human, samples of the sediment, water, fish and chicken gut were collected at a non-intensive aquaculture farm in southern China. 30 metagenomic data sets from local human gut were downloaded from the database. The antibiotic resistance genes (ARGs), mobile genetic elements (MGEs) and their hosts were identified, thus to reveal their co-occurrence, meanwhile to track the ARGs sources.

Findings/Results: The results demonstrated that ARGs were widely present in the non-intensive aquaculture system and the multidrug type was most abundant accounting for 34%. ARGs of non-intensive aquaculture environment were mainly shaped by microbial communities accounting for 51%. 6 ARGs were defined as core ARGs (top 3% most abundant with occurrence frequency > 80%) which occupied 40% of ARGs numbers in fish gut samples. The qacEdelta1 and sulfonamides resistance gene sul1 formed a stable combination and were detected simultaneously in aquaculture environment and human. Additionally, 475 high quality metagenomic assembled genomes (MAGs) were recovered and 81 MAGs carried ARGs. The multidrug and bacitracin resistance genes were most abundant ARG types carried by MAGs. Strikingly, Fusobacterium_A (opportunistic human pathogen) carrying ARGs and MGEs were identified in both aquaculture system and human gut, which indicated the potential risks of ARGs transfer.

Implications/Applications: This study explores the mobility and pathogenicity of aquaculture resistomes, emphasizes the non-intensive aquaculture environment as “hidden” ARGs reservoir and indicates potential mobility of resistomes across human-environment boundaries.

Author: Yanping Mao, Shenzhen University, China

Co-Author: Tian, Li



Session A

Abstract 348

Poster Board number: 124

Genomic Comparison of Klebsiella pneumoniae Isolated from Bovine Clinical Mastitis and Human Infections

Background and Aim: Klebsiella pneumoniae is an urgent threat to public health due to the rapid development of antimicrobial resistance. Capable of causing infections in both dairy cattle and humans, K. pneumoniae may represent a significant zoonotic pathogen, though the genomic relationship between isolates from bovine and human hosts is unclear.

Procedure/Method: In this study, a comparative genomics analysis was performed using 128 K. pneumoniae sensu stricto (KpI) strains isolated from either bovine mastitis infections or human blood infections in Canada.

Findings/Results: Among these strains, 61 sequence types (STs), 54 K- locus types, and 11 O-locus types were identified, indicating high genomic diversity. Specifically, ST219 as well as various K- and O-locus types were shared between human and bovine KpI strains. Moreover, we show that KpI has a large accessory genome, consisting of ~74% of the 15852 gene clusters found, and an open pangenome. A principal component analysis of common accessory genes in each isolate revealed no clustering by host, but rather by ST, and a phylogenomic tree illustrates that human and bovine isolates can be intermixed within the same clade. Bovine KpI assemblies contain significantly more genes associated with ferric citrate iron transporters (fecIRBCD) which may provide growth advantages associated with bovine mastitis. Human KpI assemblies tend to have genes encoding additional iron acquisition systems including yersiniabactin, aerobactin, and salmochelin.

Implications/Applications: Taken together, our results suggest that bovine and human K. pneumoniae isolates are genomically similar and thus may be able to survive together within various ecological niches, allowing opportunity for genetic exchange and pathogenic expansion.

Author: Bridget O’Brien, McGill University, Canada

Co-Authors: Yushchenko, Alla; Suh, Jinha; Jung, Dongyun; Cai, Zhangbin; Nguyen, Sam; Semret, Makeda; Dufour, Simon; Ronholm, Jennifer

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 340

Poster Board number: 125

Flash Poster Presentation: S6 - Monday, May 27 - 13:45-15:30

Optimizing the Bovine Microbiome to Reduce Agricultural use of Antibiotics

Background and Aim: Bovine mastitis is one of the most common and costly diseases in the dairy industry resulting in a decreased quality and quantity of milk, expensive antibiotic-based treatments, and culling chronically infected cows. This disease causes an annual economic loss of $665 million CAD in Canada. It is an extremely complex disease and 137 different species of bacteria have been demonstrated to cause mastitis. To prevent mastitis, on average, each cow on a Canadian dairy farm receives 1.1 defined course doses of an antibiotic – including third generation cephalosporins per year. In this project we aim to define the optimal mammary microbiome composition to prevent mastitis so that probiotics can be developed to reduce or negate antibiotic use.

Procedure/ Method: We collected milk samples from 689 dairy cattle every 2 weeks for 16 months. If a cow developed a mastitis infection with Staphylococcus aureus, Escherichia coli, or Klebsiella pneumoniae each milk sample collected from that individual was analyzed via 16S rRNA targeted amplicon sequencing.

Findings/ Results: Network analysis was used to determine that the presence Aerococcus urinaeequi and Staphylococcus xylosus are negatively correlated with S. aureus mastitis infection; Aerococcus sp., UCG-005, and Lachnospiraceae are negatively correlated with K. pneumoniae infection; and that the presence of non-Aureus staphylococci, A. urinaeequi, and Serratia marcescens are negatively correlated with E. coli infections. Several A. urinaeequi and S. xylosus isolates from healthy cattle and have identified several isolates from this collection that inhibit the growth of S. aureus in co-culture.

Implications/ Applications: A probiotic product with the strains above may negate the use of antibiotics in the dairy industry. In addition, this could be a proof of principal so that additional products could be developed to negate the use of antibiotics across the agricultural sector.

Author: Jennifer Ronholm, McGill University, Canada

Co-Authors: O'Brien, Bridget; Jung, Dongyun; Park, Soyoun; Cai, Zhangbin; Nguyen, Samuel; Kurban, Daryna; Dufour, Simon

Session A

Abstract 289

Poster Board number: 126

Reduction of resistant bacteria in manure treatment across 4 countries

Background and Aim: Animal manure is utilized around the world as an easily available fertilizer. Manure treatment represents a means to manage the high quantities of this material produced during livestock production. In the JPI ARMIS project, the removal efficiency of antibiotic resistant bacteria of widely applied manure treatment techniques and combinations of techniques across countries and continents is compared. Procedure/Method: The presence and concentrations of E. coli and extended-spectrum beta-lactamase (ESBL) producing E. coli, methicillin resistant Staphylococcus aureus (MRSA), carbapenemase-producing Enterobacterales (CRE), and vancomycin resistant enterococci (VRE) were determined during composting, on farm small-scale digestion and large-scale manure treatment installations in Canada, Germany, The Netherlands and Romania with harmonized protocols.

Findings/Results: No CRE were found in any country, and VRE were only detected in the Netherlands and in Canada. ESBL and MRSA were found in raw manure from dairy, pig and veal manure, with pig manure showing slightly smaller ESBL concentrations as compared to the other animal types. MRSA concentrations, in turn, were smaller than ESBL concentrations. Composting led to a gradual reduction in E. coli over time, while digestion showed varying efficiency per farm as shown for indicator E. coli, with maximum reductions around ~3 log across all countries. Reverse osmosis as applied in large scale installations reduced E. coli by greater than 4 log (per wet weight), while biological treatment showed lower removal. MRSA and ESBL could still be found in digestate of dairy, pig and veal manure as well as in solid-products from large-scale treatment installations. MRSA and ESBL were rarely detected in final compost .

Implications/Applications: This study shows that different manure treatment techniques are all able to reduce concentrations of resistant bacteria, still, they can occasionally be detected in the final product.

Author: Heike Schmitt, National Institute for Public Health and the Environment (RIVM), Netherlands

Co-Authors: Bucur, Marcela; Chalmers, Gabhan; Glaeser, Stefanie P.; Sefeedpari, Paria; Scott, Andrew; Kämpfer, Peter; de Roda Husman, Ana Maria; Topp, Ed; Ricker, Nicole

Back to the Program

Session A

Abstract 254

Poster Board number: 127

Impact of soil type on the dissemination of antibiotic resistance genes along the soil-plant continuum

Background and Aim: Land application of organic fertilizers may increase the dissemination of antibiotic resistance genes (ARGs) in agricultural soils, posing a potential risk to human health. However, the influence of soil type on the transmission of ARGs via the manure-soil-plant pathway remains unclear. The aims of this study was to explore the profile of ARGs along the soil-plant continuum in six contrasting soil types and identify the underlying environmental drivers.

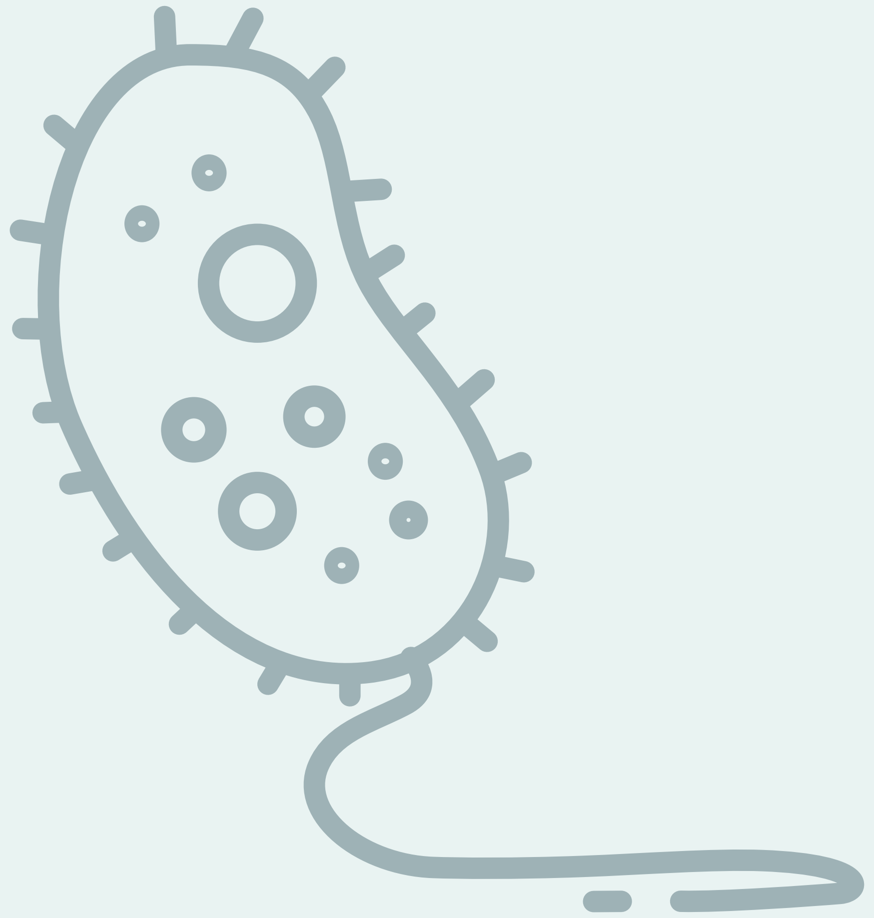
Method: A pot experiment was conducted for three months and the plant was separated into four parts: rhizosphere, root endosphere, phyllosphere and leaf endosphere. ARGs profiles and bacterial community composition were identified by high-throughput quantitative PCR and 16S rRNA gene amplicon sequencing, respectively.

Results: A total of 167 ARGs in soils and 142 ARGs in plants were detected. Aminoglycoside and multidrug resistance genes were the most abundant soil ARGs. ARGs (particularly sulfonamide resistance genes carried by the organic fertilizer) largely persisted in all soils during the entire growing period. Genes conferring resistance to multidrug, beta-lactam and macrolide-lincosamide-streptomycin B (MLSB) were dominant in the plants with fewer ARGs detected in the interior compartments (root and leaf endosphere) compared to the exterior (rhizosphere and phyllosphere). Multivariate analysis of variance indicated that the soil type significantly affected ARG distribution in soil and plants. Manure-derived organic fertilization significantly altered the ARG profile in soils, but had no significant effect on their distribution in plants. Bacterial community composition and mobile genetic elements (MGEs) were identified as biotic factors directly influencing ARG distribution.

Implication: Our results highlight the strong impact of soil type on the dissemination of ARGs in soil-plant systems and provide a comprehensive understanding of ARG dynamics to mitigate the risk to human health within the One Health framework.

Author: Yaqiong Song, Research Center of Eco-Environment Sciences, Chinese Academy of Sciences, China

Co-Authors: Qiao, Min; Brandt, Kristian; Zhu, Yong-Guan



Session A

Abstract 404

Poster Board number: 128

Flash Poster Presentation: S6 - Monday, May 27 - 13:45-15:30

A Systematic Review on Swine Pathogens in China: Trends, Prevalence, and Implications for Public Health and Biosecurity

Background and Aim: Despite the critical implications of infectious diseases for animal health, human health, and economic productivity, there is a lack of a comprehensive national-scale review on swine pathogens in China. This study aims to analyze publication trends related to swine pathogens, presuming that these trends may indicate the circulation of swine pathogens in the country, with a primary focus on characterizing their temporal and geographic patterns.

Method: Following PRISMA guidelines, the study examined 7,268 articles across PubMed, Web of Science, and CAB Abstracts databases, ultimately including 998 articles. Extracted data included location, sampling site, farm size, pathogen name and strain, production stage, health status, sample collection time, diagnostic method, reported prevalence, and total sample number. Although the project also analyzed virus and parasite studies, this abstract focused on bacterial pathogens (121 articles). We utilized SAS for statistical tests, forest plots, and ArcGIS Pro for visual maps.

Results: E. coli, S. suis, B. bronchiseptica, MRSA, and E. faecalis emerged as the most researched pathogens, with forest plots suggesting a slight prevalence increase over time. Due to a small sample size and notable between-study heterogeneity, statistical tests found no significant associations between prevalence and production stages, pig health status, or farm size. Notably, 10% of the studies discussed the presence of the mcr-1 gene, experiencing a nationwide decline from 2016 to 2018, plausibly linked to the colistin ban in 2017. Furthermore, among the 11 MRSA articles, livestock markets exhibited a significantly higher average prevalence compared to farms.

Implication: The increase in the prevalence of studied pathogens emphasizes the need for continuous monitoring and stringent biosecurity measures, especially with a higher MRSA prevalence in livestock markets than in farms. The decline in mcr-1 gene prevalence, likely due to the colistin ban, underscores the importance of regulatory interventions against antimicrobial resistance.

Author: Thanaporn Thongthum, University of Florida, United States

Co-Authors: Anderson, Benjamin; Zhou, Kaiyu; Wang, Joanne

Back to the Program

Session A

Abstract 262

Poster Board number: 131

Hazard reduction and persistence of risk of antibiotic resistance during thermophilic composting of animal waste

Background and Aim: Animal production contributes substantially to the dissemination of antibiotic resistance genes (ARGs) in the environment. Thermophilic composting is cost-effective for reducing hazards in animal wastes. However, ARGs can still persist in composts and the underlined mechanism remains largely unclear. The current study aims to reveal the mechanism of the attenuation and persistence of ARGs during thermophilic composting of animal manure.

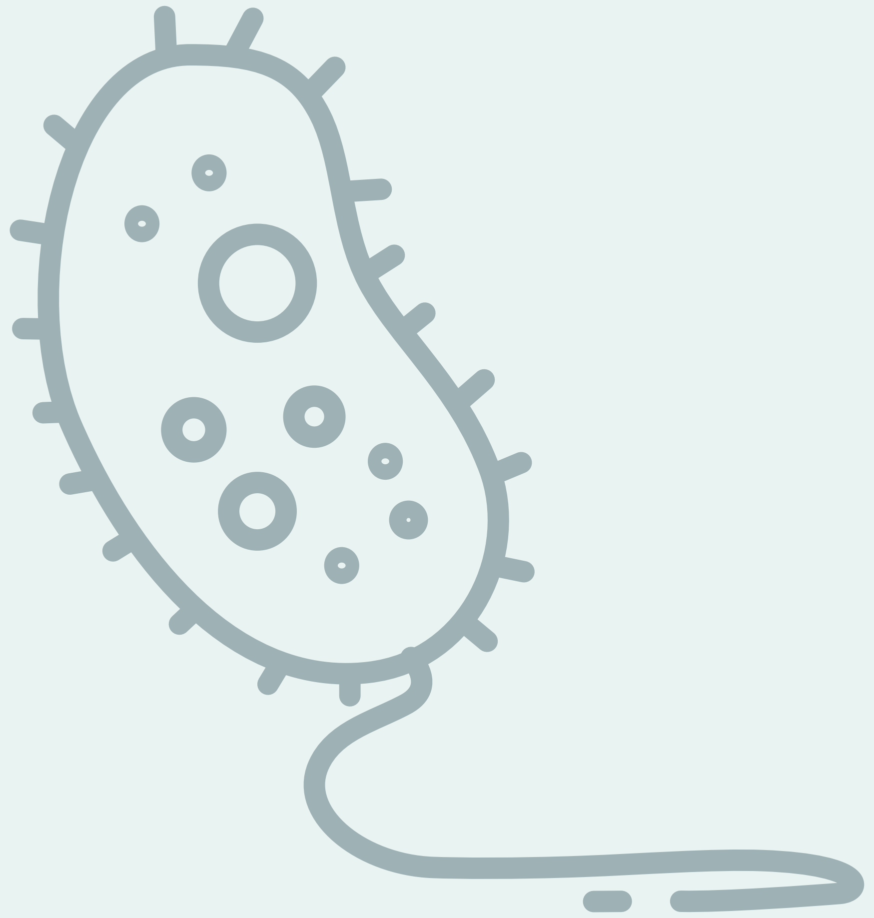
Procedure/Method: Thermophilic composting was conducted with fresh chicken feces and rice chaff. Composts on day 0, 1, 2, 4, 8, 12, 16, 21, 28, 35 and 55 were sampled for real-time quantitative PCR. Composts from Day 0, 4, 21 and 28, representing the initial material, mesophilic phase, thermophilic phase and cooling phase, respectively, were chosen for metagenomic sequencing. The analysing focus were on the dynamics of ARGs, especially those of high risk to human health, their host species and pathogenicity of the bacterial community during the composting process.

Findings/Results: Results showed that thermophilic composting was effective at reducing the majority of the spreading risk of antibiotic resistance by 1) eliminating more than half of the total ARGs that accounted for 80% of the total abundance, 2) reducing the number and abundance of high-risk ARGs significantly, and 3) removing the ARG hosts and the pathogenicity of the bacterial community substantially. The attenuation of ARGs was primarily driven by the bacterial community succession. However, the proportions of plasmid-associated ARGs and high-risk ARGs significantly increased during the composting process. In addition, the total abundances of ARGs, especially the ones associated with plasmids, collectively rebounded by 263% during the cooling phase compared to those at the thermophilic phase.

Implications/Applications: Our research reveals that minimising the horizontal transfer of plasmid-associated ARGs during the cooling phase of thermophilic composting is crucial for a more effective remediation of the antibiotic resistance.

Author: Wan-Ying Xie, Nanjing Agricultural University, China

Co-Authors: Yuan, Yi; Wang, Ya-Ting; Liu, Dong-Yang; Shen, Qirong; Zhao, Fang-Jie



Session A

Abstract 413

Poster Board number: 135

Turning One Health policy into action: Working with industry to prevent and mitigate AMR

Background and Aim: Implementing One Health AMR policy is highly challenging, because although AMR is a multi-sectoral problem that requires widespread action, many industry stakeholders have low AMR awareness and understanding. Effective engagement and support across a multitude of different industries and regulators is needed to prioritise and generate appropriate action, and to counter the propensity for AMR and its mitigation to be perceived as abstruse, too costly, or even just somebody elses's problem. This presentation will share learnings from the first phase of Australia's Cooperative Research Centre for Solving Antimicrobial Resistance in Agribusiness, Food and Environments (SAAFE CRC). This 10-year program is assisting a wide range of industries to monitor, assess, and mitigate AMR, and centres the environmental dimensions of AMR at the heart of its response. Initial projects span horticulture, viticulture, aquaculture, fisheries, water and waste.

Procedure/Method: To transition AMR from an overwhelming threat that defies action into an investment ready challenge for industries to tackle, we apply co-design principles to develop collaborative research projects with a clear pathway to translation and impact.

Findings/Results: Managing AMR is complicated by pervasive uncertainties, the multi-sectoral nature of the problem, and the complex interrelatedness of AMR risks and drivers. Well-coordinated, industry-appropriate communication is essential to support decision makers to address AMR. A conceptual action assessment framework has been developed that highlights how technological and consumer change can shift AMR from being a costly public problem to a priority challenge acted on by private enterprises.

Implications/Applications: Over the past 2 decades considerable data, information, and literature has been generated exploring the environmental dimensions of AMR, but limited behavioural and technology change has occurred. This presentation will help EDAR participants understand the barriers to change and how to increase the likelihood of their work translating into real-world impacts.

Author: Erica Donner, University of South Australia, Australia

Co-Authors: Cooper, Bethany; Crase, Lin; Jex, Aaron; Soares-Magalhaes, Ricardo; Barnes, Andrew; Ashbolt, Nicholas

Monday, May 27 - 16:00-18:00

Back to the Program

Session A

Abstract 248

Poster Board number: 137

Potential for FLOS LAMP as an Affordable Method for Quantification of Antimicrobial Resistance Indicator Genes in Low-Resource Settings

Background and Aim: Low-tech, accessible, and affordable means of environmental AMR surveillance are needed for low-resource settings. Loop-mediated isothermal amplification (LAMP) does not require a thermal cycler and provides a cost-effective, user-friendly, rapid, and sensitive alternative to PCR. The objective of this study was to develop a LAMP-based assay for detection of key AMR indicator genes, incorporating fluorescence of loop primer upon self-dequenching (FLOS) to improve specificity.

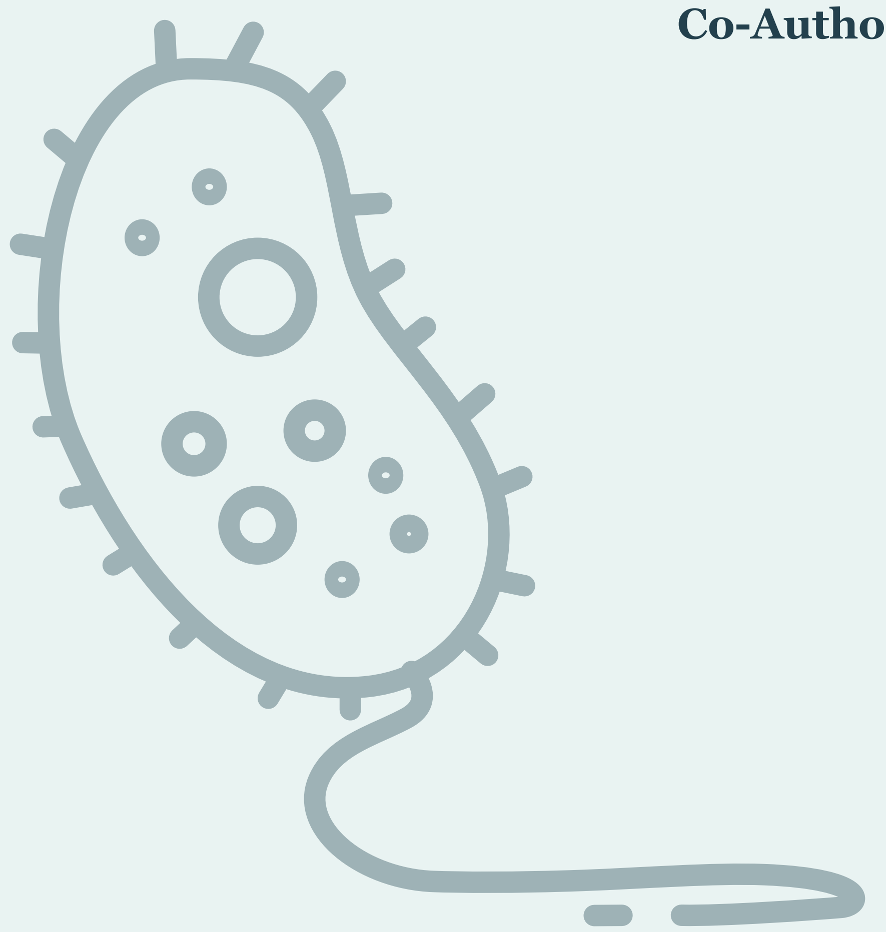
Procedure/Method: FLOS-LAMP was developed and applied to detect bla-CTX-M-1 and bla-OXA-1 ARGs and the intI1 mobile genetic element. FLOS was incorporated to improve specificity, since probes are incompatible with the unique LAMP loop-shaped secondary DNA structure. Hybridization efficiency of the FLOS probe was calculated in silico, and the FLOS probe with highest hybridization efficiency was applied. Sensitivity and specificity were assessed by comparing real-time FLOS-LAMP and droplet digital PCR (ddPCR) using plasmid stocks and wastewater samples.

Finding/Results: The FLOS probe-primer approach significantly minimized non-specific amplification while improving the detection limit and quantitative capacity relative to conventional LAMP. Improvements in R^2 of FLOS-LAMP relative to conventional LAMP were 97.96% versus 70.4% and 98.7% versus 69% for bla-CTX-M-1 and bla-OXA-1, respectively. Benchmarking to ddPCR indicated detection limits of 0.9 copies/uL for intI1 and bla-OXA-1 DNA extracts and 8 copies/uL for the bla-CTX-M-1 DNA extracts. When applied to DNA extracts from wastewater influent samples, the R^2 values were 96.3% and 89.5%, with limits of detection of 3.4 copies/uL and 11.2 copies/uL for bla-OXA-1 and intI1, respectively.

Implications/Applications: This study takes a key step towards low-cost, quantitative, and field deployable wastewater-based surveillance of AMR in low-resource settings.

Author: Petra Choi, Virginia Tech, United States

Co-Authors: Pruden, Amy; Vikesland, Peter; Kang, Seju; Scott, Katherine



Session A

Abstract 430

Poster Board number: 138

Metagenomic tools to detect emerging pathogens and antimicrobial resistance genes for wastewater-based surveillance

Background and Aim: Today, there are 1.3 million people dying annually from complications of resistant infections. It is now recognized that antimicrobial resistance (AMR) as well as the emergence of new pathogens is a global threat to animal and human health, food security, economic growth, and development. With considering of multiple source species (e.g., human and livestock species), their interactions, and their connecting environments, an integrated manner is required to develop a robust and sustainable solution for AMR surveillance. Here, we propose the development of metagenomic assays utilizing advanced, sensitive, and accurate genomic workflows with analytical pipelines to detect emerging pathogens and AMR genes.

Procedure/Method: A capture-based assay was selected with the goal of linking pathogens detected in wastewater and isolated from patients as our initiative on wastewater-based surveillance. The assay validation will be against real wastewater samples. DNA extracts from wastewater were tested with the hybridization capture assay which targets >40 viruses, >180 bacteria, >50 fungi and >1200 AMR alleles with predicted resistance to >60 antimicrobial agents.

Findings/Results: We tested wastewater samples where we were able to detect several bacteria and AMR genes showing that our newly developed metagenomic surveillance tools can provide a rapid molecular detection system to detect emerging pathogens and AMR genes present in wastewater. This will provide an early detection system allowing for early onset of outbreak investigation.

Implications/Applications: Our metagenomic tools allow for large numbers of targets and samples to be simultaneously analysed, which will expand surveillance capacity of laboratories over the handling of isolates only. The current AMR surveillance approaches are based on isolation of specific strains from clinically submitted cases. The new metagenomic assays will be able to compare genomic information recovered in a culture-free fashion from wastewater with sufficient breadth to the whole genome sequences from clinical isolates.

Author: Ju-Ling Liu, McGill University, Montreal, Quebec, Canada, Canada

Co-Authors: H.H. Djambazian, J.A.Cervantes, Sukriye Celikkol Aydin, J.A.Cervantes, Dominic Frigon, S.Dorner, J.Ragoussis

Back to the Program

Session A
Abstract 31
Poster Board number: 141

Flash Poster Presentation: S4 - Monday, May 27 - 11:00-12:30

Phenotypic Metagenomics Reveals Wastewater-Associated Rare but Clinically Important Broad-Spectrum Beta-lactam Resistance Invading River Ecosystem

Background and Aim: The emergence of clinically important antibiotic resistance in environmental reservoirs has raised serious concerns on their propagation and transmission to animals and humans. However, the high bacterial diversity and complexity of environmental microbiota makes it challenging to comprehensively quantify rare and diverse resistance determinants in environmental reservoirs.

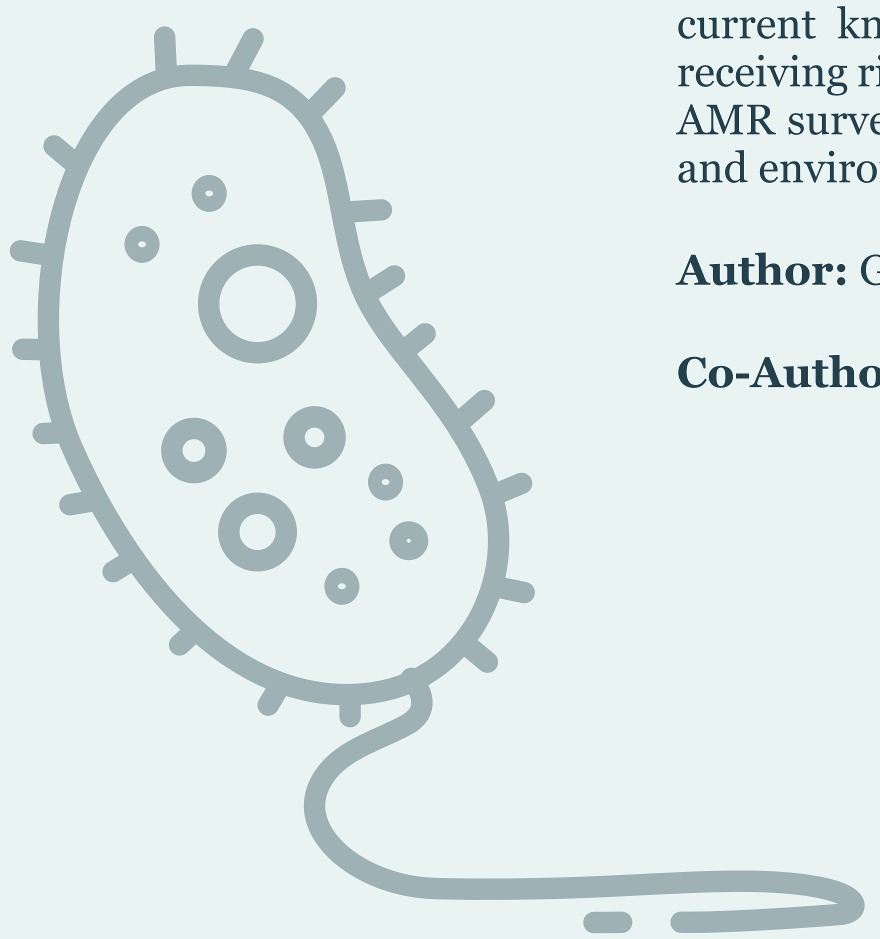
Procedure/Method: Here, we introduce phenotypic metagenomics that integrates culture enrichment, phenotypic screening, and metagenomic analyses as an emerging standardized methodology for targeted resistome monitoring and apply it to decipher the extended-spectrum β-lactam resistome in municipal wastewater treatment plants (WWTPs) and the receiving rivers in both China and Switzerland. We leverage the power of genome-centric phenotypic metagenomics for charting population-level antibiotic resistome of rare but clinically important carbapenem- or cephalosporin-resistant bacteria occurred in WWTPs and river ecosystem.

Findings/Results: The results showed that clinically prevalent carbapenemase genes (e.g., the NDM and KPC families) and extended-spectrum β-lactamase genes (e.g., the CTX-M, TEM, and OXA families) were prevalent in the WWTP and showed prominent potential in horizontal dissemination. Strikingly, carbapenem and polymyxin resistance genes co-occurred in the highly virulent nosocomial pathogens Enterobacter kobei and Citrobacter freundii. Moreover, we discovered wastewater-promoted selection of multidrug-resistant opportunistic human pathogenic bacteria in the receiving river water. Especially, epilithic biofilm was identified as an unrecognized reservoir of wastewater-associated carbapenem resistance. Our findings called for a thorough life cycle assessment of the selection and transmission of last-resort antibiotic resistance determinants from clinical reservoirs to water environment.

Implications/Applications: This study demonstrates phenotypic metagenomics as an emerging high-throughput surveillance approach of environmental resistomes and substantially expands current knowledge on extended-spectrum β-lactam resistance throughout WWTPs and the receiving river ecosystems. The methodology represents a multifaceted ‘One Health’ approach to AMR surveillance to understand and manage the threat posed to health (human, animal, plant and environmental) and security (food and water security and biosecurity).”

Author: Guoqing Zhang, Westlake University, China

Co-Authors: Ju, Feng; Buergmann, Helmut



Session A
Abstract 226
Poster Board number: 142

Enhanced taxonomic resolution for antibiotic resistance gene host bacteria identification using modified epicPCR

Background and Aim: Understanding the intricate dynamics of antibiotic resistance gene (ARG) dissemination in the environment requires precise identification of host bacteria. EpicPCR (Emulsion, Paired Isolation and Concatenation PCR) is a valuable method for pinpointing these hosts within complex microbial communities. However, conventional epicPCR, which links 288-bp 16S rRNA gene segments to functional genes, faces limitations in achieving accurate bacterial species identification. This study aims to enhance the taxonomic resolution of ARG host bacteria through a modified epicPCR approach.

Procedure/Method: Our study involved screening nested PCR primer pairing strategies, optimizing PCR conditions, and integrating PacBio long-read sequencing technology for high-fidelity results. Focusing on the oxazolidinone-phenicol resistance gene optrA, we verified the specificity and accuracy of the modified epicPCR using artificial bacterial communities. Furthermore, we evaluated its performance in swine manure anaerobic digestion systems, conducting a parallel comparison with conventional epicPCR.

Findings/Results: The optimal nested primer pairing strategy, denoted as “”inner (ARG primer)-outer (16S primer, 16S-1492R),”” fused ARGs with 974-bp 16S rRNA gene segments spanning the V4-V9 regions. Application of both conventional and modified epicPCR methods to artificial bacterial communities revealed that the modified approach reduced false positives while enhancing taxonomic resolution. In anaerobic digestion samples, the modified epicPCR demonstrated high consistency in the diversity and composition of host bacteria compared to the conventional method. Significantly, it exhibited substantial improvement in identifying amplicon sequence variants (ASVs) to the species level, especially enhanced identification of species with high abundance.

Implications/Applications: Adaptable to target any ARGs in environmental matrices, our modified epicPCR method offers enhanced taxonomic resolution crucial for identifying specific resistance reservoirs. This advancement facilitates targeted interventions, aids in surveillance of targeted pathogens, and enhances scrutiny of environmental health.

Acknowledgement:

This work was supported by National Natural Science Foundation of China (Grants 32141002).

Keywords: epicPCR, single-cell, long-read sequencing, taxonomic resolution, optrA

Author: Shihai Liu, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, China

Co-Authors: Dai, Shiting; Wang, Chen; Feng, Haodi; Han, Ziming; Yang, Min; Zhang, Yu

Back to the Program

Session A

Abstract 416

Poster Board number: 146

Whole-genome sequence of Aeromonas hydrophila CVM861 isolated from diarrhetic neonatal swine

Background and Aim: Aeromonas hydrophila are ubiquitous in the environment. They have long been known as fish pathogens but are opportunistic human pathogens. Traditionally, clinical and agriculture settings have been the primary focus of public health officials for monitoring antimicrobial-resistant microorganisms. Currently, a more integrated approach, One Health, includes the environmental microbiome, mobilome, and resistome. Whole genome sequencing of an Aeromonas hydrophila isolate potentially elucidates the possible fluidity via recombination between chromosomal and plasmid locations of virulence and resistance genes and provides information on the resistome of food production animals and the larger global resistome.

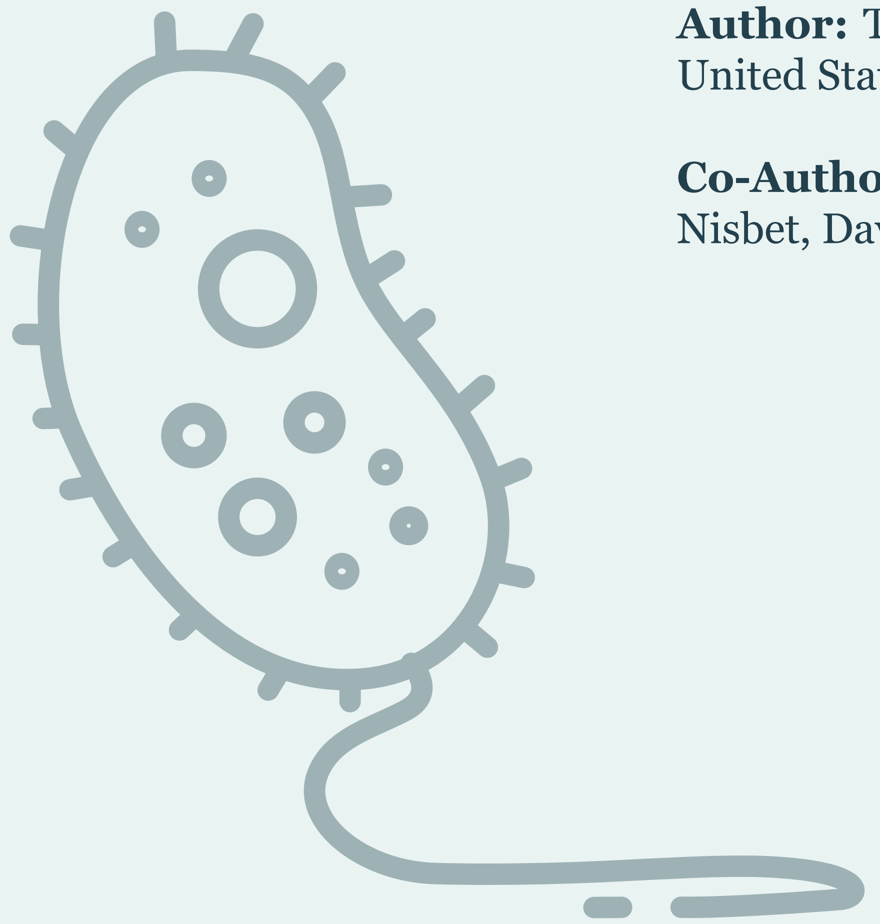
Procedure/Method: A beta-hemolytic, putative Escherichia coli strain collected from diarrheic neonatal pigs in Oklahoma was subsequently identified as Aeromonas hydrophila, and designated CVM861. Antimicrobial susceptibility testing was performed by broth microdilution according to Clinical Laboratory Standards Institute (CLSI) methods. The whole genome was sequenced and sequences were uploaded to the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) where they were assembled and checked for alignments in different databases. Contigs were also aligned with Aeromonas-specific virulence genes in Genbank.

Findings/Results: The sequence data for CVM861 revealed four Aeromonas-specific virulence genes: lipase (lip), hemolysin (hlyA), cytonic enterotoxin (ast) and phospholipid-cholesterolacyltransferase (GCAT). There were no alignments to any virulence genes in VirulenceFinder. CVM861 contained an Escherichia coli resistance plasmid identified as IncQ1_1__M28829. There were five aminoglycoside, three beta-lactam, and one each of macrolide, phenicol, sulfonamide, tetracycline and trimethoprim resistance genes, identified in the ResFinder database. Additionally, there were 36 alignments to mobile genetic elements using MobileElementFinder.

Implications/Applications: Aeromonas hydrophila as an aquatic pathogen, is rarely considered in human disease. Nevertheless, it contributes to the resistome reservoir and may be capable of transferring resistance and virulence genes to other more prevalent foodborne strains such as Escherichia coli or Salmonella in swine or other food production systems.

Author: Toni Poole, United States Department of Agriculture, Agricultural Research Service, United States

Co-Authors: Schlosser, Wayne D.; Anderson, Robin C.; Norman, Keri N.; Beier, Ross C.; David Nisbet, David Nisbet



Session A

Abstract 205

Poster Board number: 147

Flash Poster Presentation: S5 - Monday, May 27 - 13:45-15:30

Molecular epidemiology and mechanisms of carbapenem and colistin resistance in Klebsiella and Enterobacter from treated wastewater

Background and Aim: The emergence and spread of carbapenem-resistant Klebsiella spp. (CRK) and Enterobacter cloacae complex (CREC) pose a serious threat to human health worldwide. Treated wastewater can release these bacteria into the environment, potentially reintroducing them into the population. Therefore, the aim of this study is to isolate CRK and CREC isolates from treated municipal wastewater in Croatia and to determine their phenotypic and genetic characteristics.

Methods: CRK and CREC were isolated from treated wastewater using CHROMAgar mSupercarba plates. The isolates were identified by MALDI-TOF MS. Carbapenemase production and antibiotic resistance profiles were analyzed phenotypically. Carbapenemase and mcr genes were identified by PCR, while the epidemiological potential was assessed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). A subset of isolates with concomitant carbapenem and colistin resistance phenotype (8 CRK and 3 CREC) was selected for in-depth whole-genome sequencing (WGS) analysis.

Findings: Among 82 isolates, 47 were identified as CRK and 35 as CREC. All isolates were carbapenemase producers with multi-drug resistant phenotype. Colistin resistance was observed in 40% CRK and 57% CREC. PCR analysis predominantly identified blaNDM-1, and blaOXA-48 in CRK, while blaKPC-2 frequently occurred in CREC. Fifteen sequence types (ST) were identified, 12 of which were human-associated clones. WGS revealed various plasmids, resistance genes, and virulence genes. Additionally, sequenced CRK isolates harbored potentially novel, and CREC isolates known intrinsic mechanisms for colistin resistance (point mutations in the pmrA/B, phoP/Q, mgrB, and/or crrB genes). The acquired colistin-resistance gene mcr-4.3 was found for the first time in K. pneumoniae ST629 and on the conjugative IncHI1B plasmid.

Implications/Applications: This study demonstrates the important role that treated wastewater plays in harboring and spreading human-associated Klebsiella spp. and E. cloacae complex that are resistant to last-line antibiotics, highlighting the need for more effective wastewater treatment.

Author: Ana Puljko, Ruđer Bošković Institute, Croatia

Co-Authors: Dekić Rozman, Svjetlana; Jelić, Marko; Barišić, Ivan; Udiković-Kolić, Nikolina

Session A

Abstract 50

Poster Board number: 149

Flash Poster Presentation: S4 - Monday, May 27 - 11:00-12:30

Proximity-guided metagenomics to unravel physical association between plasmid-borne antimicrobial resistance genes and hosts in wastewater in Vietnam

Vietnam has faced a growing threat from antimicrobial resistance, resulting in the apparent health risk caused by ESKAPE pathogens in clinical settings. Only 12.5% of urban wastewater is currently treated before discharge to the environment. Thus, it is likely that the release of untreated wastewater contributes to the spread of antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) into the environment in Vietnam. However, ARB and ARGs contained in urban wastewater in Vietnam have not been fully characterized. In particular, it is challenging to explore hosts of mobile ARGs on plasmids in complex bacterial communities in the environment. In this study, we aimed to apply proximity ligation (Hi-C) analysis to reveal physical association between mobile ARGs on plasmids and their hosts in wastewater. Proximity ligation sequencing is a powerful culture-independent technique that captures physical associations between plasmid-borne ARGs and their hosts in complex microbial communities. Urban wastewater samples collected from Hanoi and Ho Chi Minh City in 2023 were analyzed. In addition to the analysis of original wastewater samples, they were cultivated with antimicrobial agents (cefotaxime, meropenem, colistin, and tigecycline) to enrich minor ARB in the samples. High-quality metagenome-assembled genomes (MAGs) (completeness > 70% and marker gene overrepresentation < 10%) were extracted and associated plasmids carrying ARGs were determined. MAGs with plasmid-borne ARGs conferring resistance to macrolide, tetracycline, sulfonamide, etc., were obtained from the original wastewater. Interestingly, some MAGs were affiliated with genome sequences which show little homology to the known ones. This indicates a great diversity of currently unknown resistance reservoirs in wastewater. From the samples selected with meropenem, for instance, a Pseudomonadales-affiliated MAG was associated with a plasmid carrying blaIMP-1 encoding carbapenemase. We show that this approach can be used to establish a comprehensive database of environmental ARB with their associated repertoire of plasmidome and resistome elements.

Author: My Hanh Vu Thi, The University of Tokyo, Japan

Co-Authors: Kurisu, Futoshi; Suzuki, Yuji; Hasebe, Futoshi; Glidden, Demi; Liachko, Ivan; Suzuki, Masato; Kasuga, Ikuro

Session A

Abstract 251

Poster Board number: 151

Flash Poster Presentation: S17 - Thursday, May 30 - 11:00-12:30

Methane and Nitrous Oxide Emissions from Manure: Effects of Antibiotic Residues

Background and Aim: Manure is an excellent source of nutrients and is used to amend soils, but improper management can have negative environmental effects like the release of greenhouse gases (GHG), nutrient-related water resource pollution, antimicrobial residues, and antimicrobial resistance (AMR). We investigated the effects of three commonly used antibiotics in livestock manure on GHG emissions.

Method: Fresh cattle manure (10 kg) was spiked with 100 mg kg-1 (low), 700 mg kg-1 (high) tylosin, enrofloxacin 29 mg kg-1 (low), 200 mg kg-1 (high), and oxytetracycline 229 mg kg-1 (low) and 1600 mg kg-1 (high) dry weight manure and incubated aerobically in 40 L barrel-shaped plastic containers to simulate the heaping method commonly practiced by cattle farmers in Sub-Saharan Africa. The control experiment had the same conditions but was antibiotic-free. CH4 and N2O emissions flux from the manure were measured daily during the first month and thrice weekly using static chamber method with a Picarro G2508 model for 47 days to assess the cumulative emissions of methane and (CH_4) nitrous oxide (N_2O) released per unit of treated manure.

Findings: Manure heaps act as great sources of CH4 and N2O. Tylosin (low) and enrofloxacin (high) increased cumulative N2O emissions significantly by 221.65%, and 156.72% respectively compared to the control. Methane emissions were increased by low concentrations of enrofloxacin and tylosin by 210.82% and 213.37 % respectively. Both concentrations of oxytetracycline did not affect either CH4 or N2O emissions. Our findings provide new knowledge on the environmental trade-off associated with using antibiotics in livestock in Sub-Saharan Africa.

Implications: Antibiotic residues in manure can alter the decomposition process during manure storage, impacting GHG emissions and nutrient content. Moreover, manure containing antibiotic residues applied to fields as a fertilizer can leach into the soil and water, disrupting various biological processes, such as soil nutrient cycling.

Author: Chebet Arusey, International Livestock Research Institute (ILRI), Kenya

Co-Authors: Moodley, Arshnee; Leitner, Sonja; Muloi, Dishon; Ng'etich, Wilson; Kebeney, Syphyline; Yuhao, Zhu; Oduor, Collins

Back to the Program

Session A

Abstract 242

Poster Board number: 153

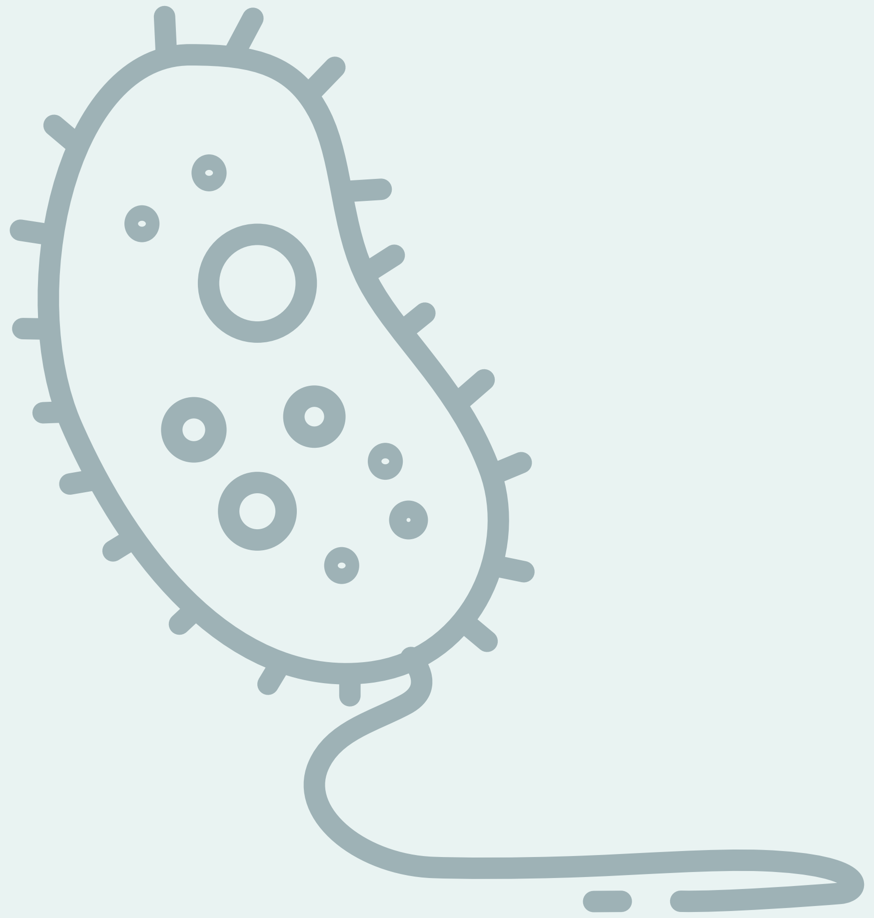
Antimicrobial Resistance, Environmental Racism & Climate Change: A Deadly Triangle

Background: Health disparities stemming from environmental racism have put marginalized communities at increased risk of contracting antimicrobial-resistant (AMR) diseases. These communities have faced disproportionate exposure to environmental hazards like air pollution and waste, negatively impacting their health and well-being.

Findings: Historically redlined communities bear the brunt of environmental injustices, experiencing exceedingly high levels of pollution, limited access to healthy food options, and inadequate sanitation. These factors, along with chronic health conditions like asthma and diabetes, compromise residents' immune systems and make them significantly more susceptible to AMR infections. Climate change will further intensify these disparities, creating favorable conditions for the spread of antibiotic-resistant bacteria. Extreme weather events like heat waves, floods, and wildfires will become increasingly frequent and severe, disproportionately impacting vulnerable communities. Population displacement during natural disasters often leads to overcrowded conditions in temporary shelters, increasing infection rates and the transmission of infectious airborne diseases. Disruptions to vaccinations and other preventative medicines during these events can lead to a higher prevalence of preventable diseases. Additionally, natural disasters like earthquakes and major flooding disrupt access to clean water and sanitation, further increasing the risk of waterborne diseases and facilitating the spread of AMR pathogens.

Implications: The intertwined threats of environmental racism, climate change, and AMR demands urgent collective action. Policymakers, healthcare professionals, and community leaders must collaborate to ensure equitable access to healthcare, clean air and water, and investments in sustainable infrastructure within marginalized communities. This is crucial to mitigating the impact of AMR and protecting the health of all populations, particularly those most vulnerable. Policy changes, improved healthcare access, reduction of pollution, and investments in infrastructure are crucial steps in safeguarding the health of vulnerable communities. Policymakers must recognize the urgency of this issue and work collaboratively with these communities to ensure a more equitable and sustainable future.

Author: Zanah Francis, HHS, United States



Session A

Abstract 63

Poster Board number: 156

Flash Poster Presentation: S3 - Monday, May 27 - 11:00-12:30

An analysis of wastewater antimicrobial resistance genes before and after the lifting of COVID-19-related travel restrictions

Background and Aim: Travel has been recognized as a key mechanism of antimicrobial resistance (AMR) dissemination. Among the measures undertaken to reduce COVID-19 spread, the Canadian government introduced a temporary travel ban. These measures were then removed during the transition to the endemic phase of COVID-19, creating an opportunity to assess if AMR determinants changed. This study surveyed AMR genes in wastewater at a range of scales before and after the lifting of COVID-related restrictions.

Procedure/Method: Untreated 24-hour composite wastewater samples were collected longitudinally across a range of scales, including a wastewater treatment plant (representing the whole community), university campus residential and non-residential buildings, and a homeless shelter in the 7 months before and after the restrictions were lifted on Mar-01-2022. Genomic DNA was extracted from the wastewater, and Illumina sequencing was followed by DeepARG-SL (v2.0) analysis. We used Two-Way ANOVA to compare AMR gene abundance before and after the lifting of restrictions.

Findings/Results: Abundances of 221 out of 439 AMR genes, including clinically important genes such as OXA, TEM, CARB, qnrS, and ermB, varied both by time period and location ($p<0.05$). Specifically, AMR genes increased significantly after the lifting of COVID-related measures across all sites. The analysis also uncovered a significant interaction between two variables (time period and location) ($p<0.05$). Specifically, the magnitude of the increase in AMR genes in the months following the lifting of travel restrictions depended on the type of site.

Implications/Applications: Our results demonstrate that the burden of AMR genes across a range of scales in Calgary increased after the lifting of COVID-19 restrictions. Levels of change varied significantly by population group, underscoring the importance of both community and granular monitoring. Our results suggest that travel is a potential driver of AMR in Calgary across a range of scales, and warrants further study.

Author: Jangwoo Lee, University of Calgary, Canada

Co-Authors: Gonzalez, Catalina; Au, Emily; Xiang, Kevin; Du, Kristine; Waddell, Barbara; Acosta, Nicole; Bautista, Maria; Bhatnagar, Srijak; Lee, Bonita; Pang, Xiaoli; Weyant, Benson; Pitout, Johann; Rennert-May, Elissa; Conly, John; Hubert, Casey; Parkins, Michael

Back to the Program

Session A

Abstract 252

Poster Board number: 158

A vision for metagenomic-enabled environmental AMR surveillance that is adaptive to low- and high-income settings and water infrastructure constraints

Background and Aim: Antimicrobial resistance (AMR) is a global threat that merits urgent coordinated surveillance to inform policy and practice to combat its spread. Challenges include balancing cost with selection of monitoring sites and targets across One Health sectors, as well as storing and adequately analyzing data produced. Here we propose a vision for achieving this goal, drawing from experiences and lessons learned from a series of international sampling campaigns initiated by the Halting Environmental Antimicrobial Resistance Dissemination (HEARD) collaborative.

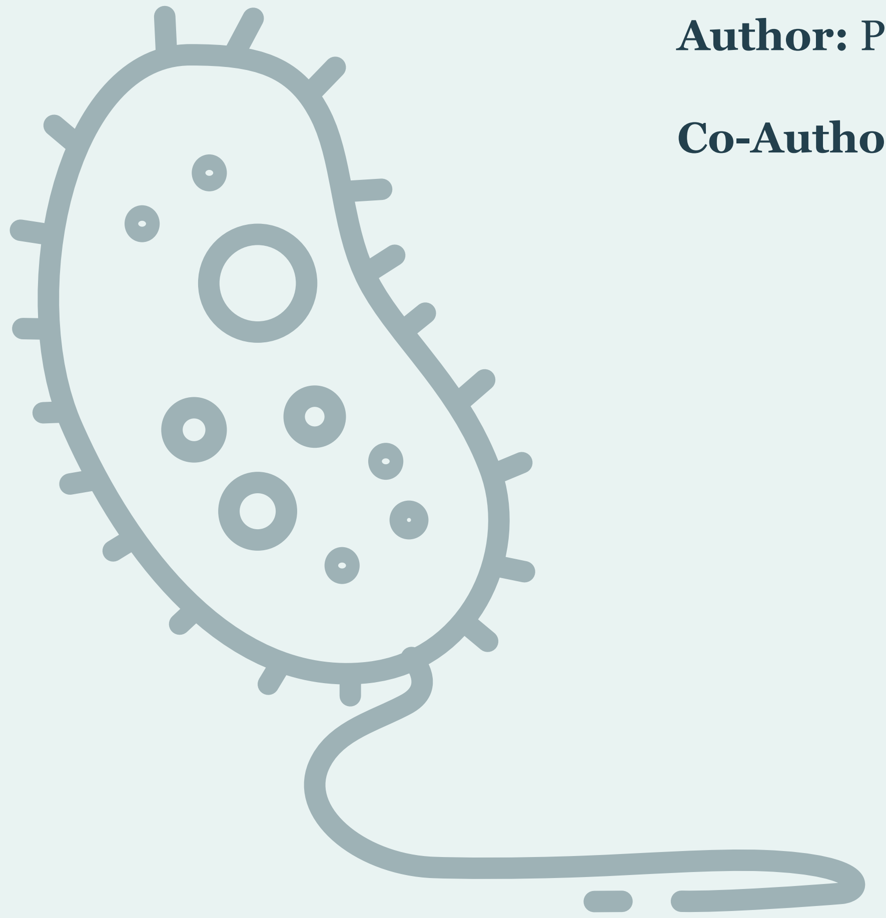
Procedure/Method: Starting in 2015, samples subjected to shotgun metagenomic sequencing and antibiotic profiling were collected from multiple stages of wastewater and water reuse treatment plants and up/downstream aquatic receiving environments. The first suite of sampling included locations in India, Hong Kong, Philippines, Sweden, Switzerland, and USA, with a follow-up campaign carried out in summer 2023 to expand sampling in these locales and to also include Portugal and Botswana. The resulting data are being used as a training ground for interdisciplinary students specializing in environmental science/engineering, computer science/bioinformatics, and policy as a means to develop tools and approaches to relate monitoring data to relevant environmental and socioeconomic factors.

Findings/Results: The HEARD collaborative has catalyzed development of a cyberinfrastructure for analysis of environmental metagenomes, including tools for quantifying and comparing antibiotic resistance genes (ARGs), mobile genetic elements, and pathogen markers. Key trends have been identified through application of these tools that have important policy implications. These include relationships between wastewater/aquatic ARG profiles and antibiotics measured in sewage, antibiotic use policy and practice, socioeconomic status, size of the population served, infrastructure status, and historical implementation of AMR policy.

Implications/Applications: This study proposes a framework for metagenomics-enabled integrated AMR surveillance of wastewater and affected aquatic environments that is relevant to a range of socioeconomic and infrastructure scenarios, including high- and low-income settings.

Author: Peter Vikesland, Virginia Tech, United States

Co-Authors: Pruden, Amy; Zhang, Liqing; Brown, Connor; Blair, Matthew



Session A

Abstract 104

Poster Board number: 160

What about outside the cell? Abundance and diversity of intracellular and extracellular ARGs in urban stormwater and surface waters

Background and Aims: Research that quantifies ARGs in environmental systems has often ignored the contribution of extracellular ARGs (eARGs) to the environmental resistome. The objective of this research was to fill this gap through a robust monitoring study that quantified the abundance and diversity of iARGs and eARGs in environmental aquatic samples.

Method: Quantitative analysis of iARGs and eARGs was completed for five key environmental locations: (1) drinking water, (2) stormwater, (3) river surface water, (4) lake surface water, and (5) wastewater effluent. Four ARGs were quantified along with the mobile genetic element (MGE) intI1. Following, whole genome metagenomic sequencing was completed for the stormwater and river and lake surface water samples. Comprehensive analyses were performed to determine the relative abundance of iARG and eARGs in the genome, as well as the association with MGEs and the microbial community.

Findings: The relative abundance of eARGs and e-intI1 were not significantly different from each other in the river, lake, stormwater, and wastewater effluent samples. Interestingly, there were many instances in which the extracellular abundance was either greater or not significantly different than that of the intracellular abundance. Metagenomic analysis revealed that the iARG and eARGs had similar levels of diversity in each environmental sample but were distinctly different in composition.

Implications: These results confirm that eARGs are an overlooked, yet relevant component of environmental resistomes. They are relevant for the proliferation of antibiotic resistance and the interactions that lead to gene persistence. Furthermore, the difference in diversity between the intracellular and extracellular resistomes indicates that, in comprehensively characterizing ARGs in the environment, eARGs must be included.

Author: Kassidy O’Malley, Marquette University, United States

Co-Authors: McNamara, Patrick; McDonald, Walter

Back to the Program

Session A

Abstract 112

Poster Board number: 161

Microbiome Diversity and Biosafety of Digestate Derived from Agricultural Biogas Plants in Circular Waste Management

Background and Aim: The composition of microbial communities, including Bacteria and Archaea, is the key to effective anaerobic digestion (AD). The microbiome driving the AD process has been extensively researched, whereas the influence of specific substrates on the microbiome of digestate remains insufficiently investigated. Digestate is generally regarded as a waste product, but it has considerable potential for use in soil fertilization and bioremediation, which is why the biological safety of digestate and the composition of its microbial community should be evaluated.

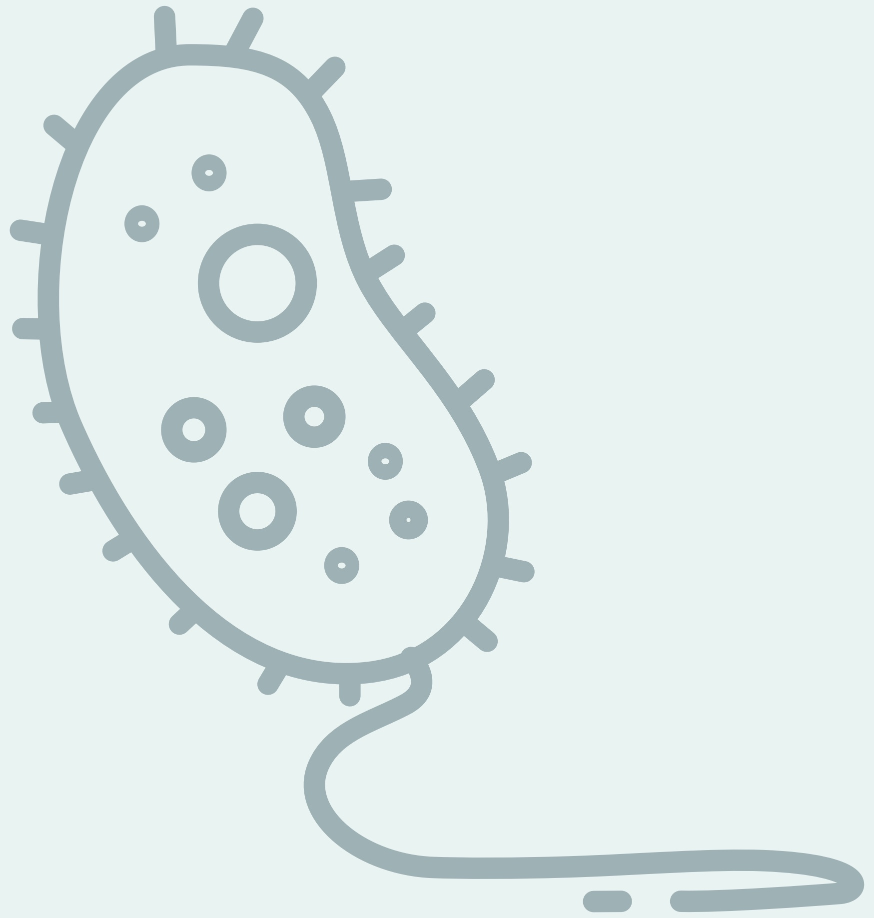
Methods: In the present study, digestates from three full-scale agricultural biogas plants were analyzed to determine the impact of substrate composition (including plant- and animal-based materials, and industrial waste) on the structure of their microbial communities. The microbial biodiversity of AD samples were analyzed using 16S rRNA gene sequencing methods. Moreover, the presence/number of Yersinia enterocolitica and Salmonella spp. bacteria and live eggs of intestinal parasites of the genera Ascaris, Trichuris, and Toxocara were also analysed.

Results: The metagenomic analysis of 16S rRNA amplicon revealed that Firmicutes and Bacteroidota were the dominant bacterial phyla (>28% and 21%, respectively) in digestates regardless of the composition of the processed substrates. However, an analysis of the Bray-Curtis dissimilarity index revealed that each digestate sample had a unique microbial composition, which indicates that digestate may have an immense fertilizing and bioremediation potential that has not been fully utilized to date. Moreover, the study also confirmed the biological safety of digestates sampled from three biogas plants in different seasons of the year. All tested digestates were free of potentially pathogenic microorganisms such as Salmonella spp. and Yersinia enterocolitica, as well as parasites such as Ascaris spp., Trichuris spp., and Toxocara spp.

Implications: These findings indicate that digestate is a valuable and safe resource that can be applied in soil fertilization and bioremediation.

Author: Ewa Korzeniewska, University of Warmia and Mazury in Olsztyn, Poland

Co-Authors: Wolak, Izabela; Harnisz, Monika; Męcik, Magdalena



Session A

Abstract 336

Poster Board number: 162

Eradication of Staphylococcus aureus Biofilms and Antibiotic-Tolerant Persisters by Enantiomeric Glycosylated Cationic Block Co-Beta-Peptides

Background and Aim: The escalating challenge of antimicrobial resistance in bacteria poses a severe threat, depleting the arsenal of therapeutic options and constraining treatment choices. Further complicating the management of drug-resistant strains is the existence of persisters, sub-populations exhibiting drug tolerance due to metabolic inactivity, and the ability of bacteria to form biofilms. Methicillin-resistant Staphylococcus aureus (MRSA) stands out as a high-priority pathogen, according to the World Health Organization (WHO). MRSA is also found in livestock, for example pigs, and has raised concerns about the impact of their animal reservoirs to public health. In this context, our objective is to develop a novel antimicrobial agent capable of effectively combating various MRSA strains, the biofilms, and the persisters, with the added goal of preventing the onset of bacterial resistance.

Procedure/Method: We develop an enantiomeric block co-beta-peptide, poly(amido-D-glucose)-block-poly(beta-L-lysine), with high yield and purity by one-shot one-pot anionic-ring opening (co)polymerization.

Findings/Results: The co-beta-peptide exhibits bactericidal activity against methicillin-resistant Staphylococcus aureus (MRSA), targeting replicating, biofilm, and persister bacterial cells, while also effectively dispersing biofilm biomass. This copolymer demonstrates surfactant-like properties activated by bacteria upon contact with the bacterial envelope. It remains effective against both community-acquired and hospital-associated MRSA strains resistant to multiple drugs, including vancomycin and daptomycin. Importantly, it does not induce bacterial resistance.

Implications/Applications: In MRSA mouse and human ex vivo skin infection models, its antibacterial efficacy surpasses that of vancomycin, and there is no observable acute in vivo toxicity in mice even with repeated dosing at therapeutic levels. These findings suggest that this non-toxic molecule, effective against various bacterial sub-populations, holds promising potential for the treatment of MRSA infections.

Author: Mary Chan-Park, NTU, Singapore

Back to the Program

Session A

Abstract 337

Poster Board number: 163

Flash Poster Presentation: S6 - Monday, May 27 - 13:45-15:30

Novel Cationic Antibacterial Polymers as Potential Antibiotic Replacements

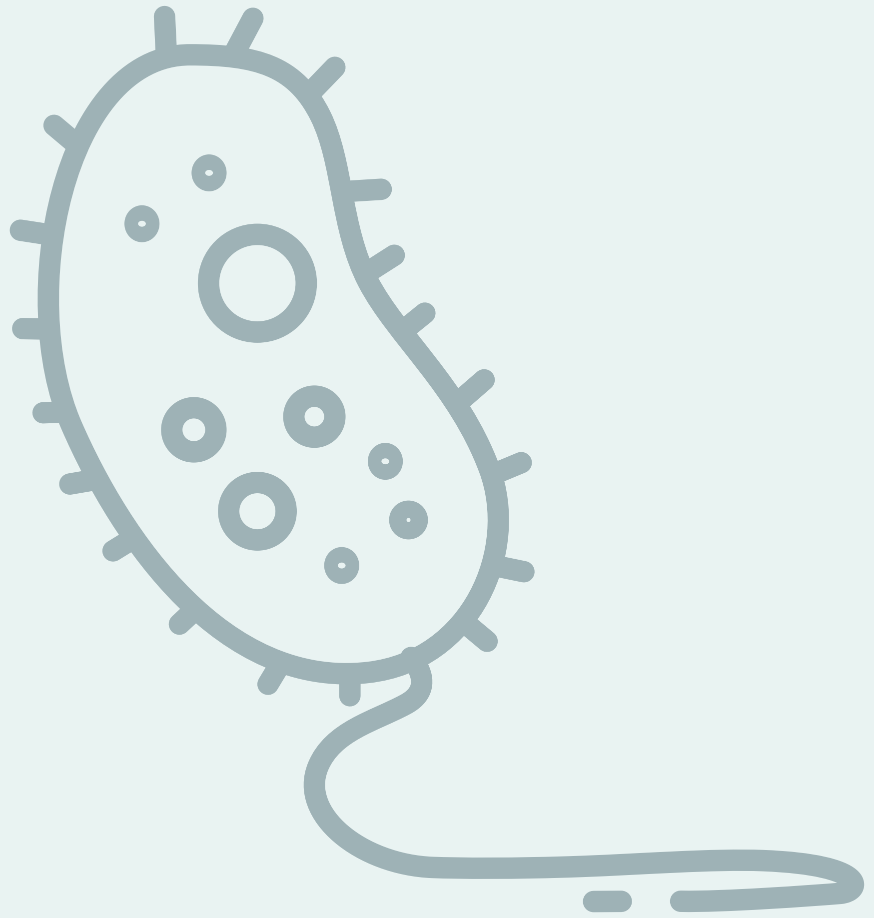
Background: Drug resistance in pathogenic bacteria is a growing concern worldwide, posing a critical threat to global health. While conventional antibiotics still play a crucial role in treating bacterial infections, the emergence and spread of antibiotic-resistant micro-organisms are rapidly eroding their usefulness. Furthermore, antibiotics are largely ineffective against persisters and biofilms as they normally target metabolically active bacteria. Biofilm bacteria can be up to 1000 times more resistant to antibiotics than their planktonic forms.

Methods: Novel antimicrobial beta-peptides featuring a distinctive block copolymer architecture are synthesized and comprehensively evaluated for their antimicrobial and antibiofilm effectiveness. Additionally, an innovative design involving imidazolium-based charge-on-backbone polymers is explored to introduce fresh perspectives in antimicrobial polymer development.

Results: A glycosylated cationic co-beta-peptide demonstrates significant antibiofilm efficacy against Gram-positive Methicillin-resistant Staphylococcus aureus (MRSA). Its robust antibiofilm activity is attributed to the combined effects of bactericidal, anti-persister, and biofilm dispersal capabilities. Additionally, a main-chain cationic polyimidazoliums (PIM) exhibits broad-spectrum antimicrobial activity, even against pan-antibiotic-resistant Gram-negative and Gram-positive bacteria, employing a novel mechanism. Moreover, this polymer exhibits intriguing efficacy against non-tuberculosis mycobacteria (NTM).

Conclusions: Innovative polymer designs, such as block copolymers and charge-on-backbone polymers, along with a novel charge center (imidazolium instead of the conventional ammonium), have the potential to expand the structure-activity relationship (SAR) window for cationic polymers. This expansion can result in compelling antimicrobial properties with a favourable safety profile.

Author: Mary Chan-Park, NTU, Singapore



Back to the Program

Session B

Abstract 397

Poster Board number: 1

Spatiotemporal surveillance of antimicrobial resistance from wastewater treatment plants in rural West Virginia (USA)

Background and Aim: Antimicrobial resistance (AMR) is a pressing issue for public health across the globe, and may be especially concerning for rural, low-income settings which typically have limited AMR monitoring and substantial barriers to healthcare access. This study leverages wastewater surveillance across the rural state of West Virginia (USA) to determine: a) trends in detection and abundance of select AMR targets, b) spatial designation of select AMR targets within specific communities and regions of the state, and c) community healthcare and wastewater infrastructure characteristics that drive abundance of detected AMR.

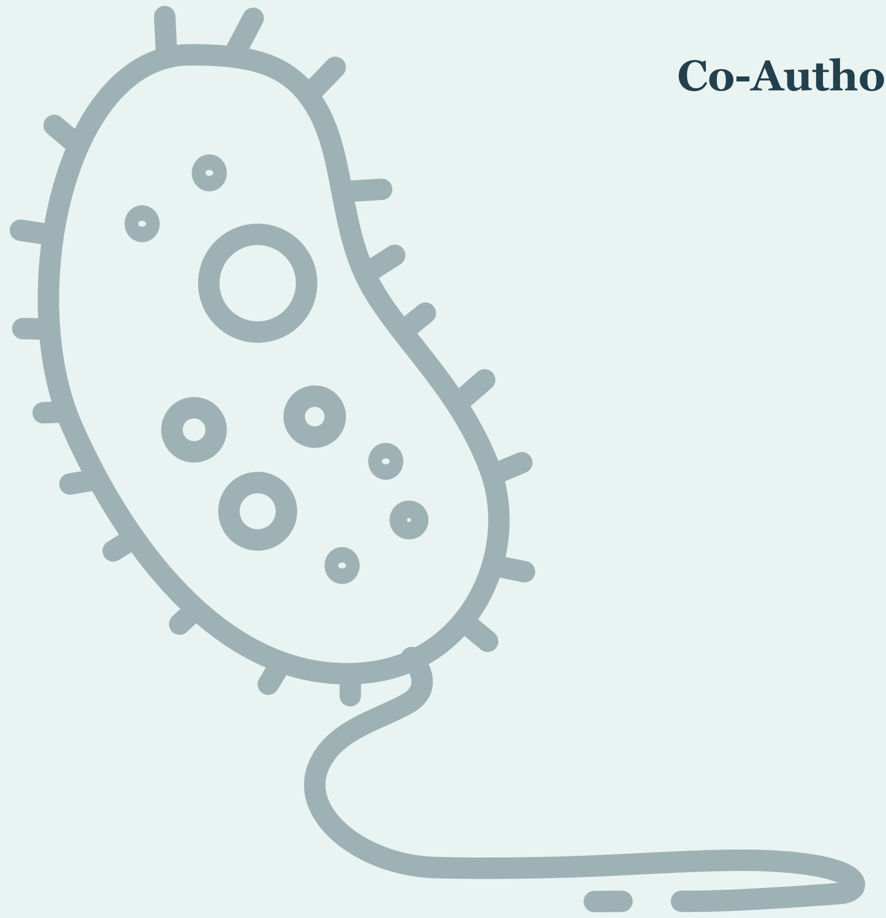
Procedure/Method: In conjunction with the Wastewater Testing for Community Health in West Virginia (WaTCH-WV) initiative, routinely collected wastewater influent samples were analyzed on a weekly basis from approximately 20 wastewater treatment plants (WWTPs) for a suite of clinically relevant antibiotic resistance genes (ARGs), including the minimally redundant wastewater indicators oqxA, ermB, sul1, and mexE, using quantitative polymerase chain reaction (qPCR).

Findings/Results: Sample collection began in December 2023 and is ongoing for one full year through November 2024. Preliminary results from the first five months of this study will be presented, indicating temporal variability of AMR loading. Spatial analytics will be used to assess topological influences on rural AMR profiles, highlighting the effects of healthcare facilities, social indices, and population metrics.

Implications/Applications: This investigation utilizes a One Health approach via wastewater surveillance paired with sociodemographic analyses to describe the impact of community healthcare access and wastewater infrastructure on detectable AMR loadings to rural WWTPs. Findings from routine monitoring will be communicated with health officials and wastewater operators to inform regional public health interventions. As a mostly rural state, the robust data generated from this study in West Virginia will accentuate AMR management strategies for small centralized systems across the Appalachian region and other vulnerable locations worldwide."

Author: Christopher Anderson, Wadsworth Dept. of Civil and Environmental Engineering at West Virginia University, United States

Co-Author: Garner, Emily



Session B

Abstract 214

Poster Board number: 4

Towards the development and evaluation of a sewage surveillance system to predict antibiotic resistance in Klebsiella pneumoniae from humans

Background and aim: Recent studies have shown promise in predicting clinical antibiotic resistance rates from sewage data. Few have focused on Klebsiella pneumoniae despite its importance as a carrier of antibiotic resistance. Here, we aimed to suggest an efficient culture protocol for isolation of K. pneumoniae from sewage and to compare collected sewage isolates with clinical data.

Method: Using sewage samples from Gothenburg, Sweden, ten culture conditions were evaluated in parallel: five agars incubated at 37˚C or 42˚C. Colony counts and MALDI-TOF mass spectrometry analysis of collected presumed K. pneumoniae isolates were used to assess sensitivity and precision. Resistance rates were determined using broth screening and disk diffusion. To more efficiently capture and assess the prevalence of ESBL-producing K. pneumoniae, thirteen hospital sewage samples collected over a year were cultured on SCAI agar plates supplemented with cefpodoxime-cloxacillin. ESBL production was verified through Double Disk Synergy Test.

Results: The sensitivity was similar for most media irrespective of incubation temperature. For four media, a better precision was observed after incubation at 42˚C. SCAI media incubated at 42˚C showed the highest precision (84.4%). K. pneumoniae isolates from municipal sewage showed overall considerably lower resistance rates than isolates from clinical samples, potentially reflecting the resistance prevalence in gut K. pneumoniae of the local population. Resistance screening of hospital sewage isolates has just been initiated. However, direct plating showed that relative ESBL prevalence was significantly higher in hospital sewage compared to municipal sewage. Results from genetic comparisons with ESBL isolates from local patients will be presented at the conference.

Implications: This study could contribute to the development and evaluation of a resource-efficient sewage-based surveillance system targeting antibiotic resistance in K. pneumoniae, which could be particularly valuable in parts of the world with limited clinical surveillance.

Author: Julián Bobis, Centre for Antibiotic Resistance Research in Gothenburg (CARe); Institute of Biomedicine, Department of Infectious Diseases, University of Gothenburg, Sweden

Co-Authors: Nilsson, Johanna; Åhrén, Christina; Larsson, D. G. Joakim; Flach, Carl-Fredrik

Back to the Program

Session B

Abstract 165

Poster Board number: 5

GPS-tagged Black-headed gulls give insight into the dispersal of Carbapenemase-producing Enterobacterales from WWTP:s

Background and Aim: The role of wildlife in the dispersal of clinically relevant antimicrobial resistance (AMR) is not fully understood. PAIRWISE is an international JPIAMR project focusing on the dispersal of antibiotic resistance determinants and antibiotic resistant bacteria in aquatic ecosystems and its impact on associated wildlife and livestock. In this study we determined whether wildlife provide a dissemination pathway for AMR. Using Black-headed gull (Chroicocephalus ridibundus) foraging at wastewater treatment plants (WWTPs) as a candidate vector species, we characterized and quantified AMR at the WWTP, at nesting colonies, and sensitive environments identified through the tracking of individual gulls using GPS-tags.

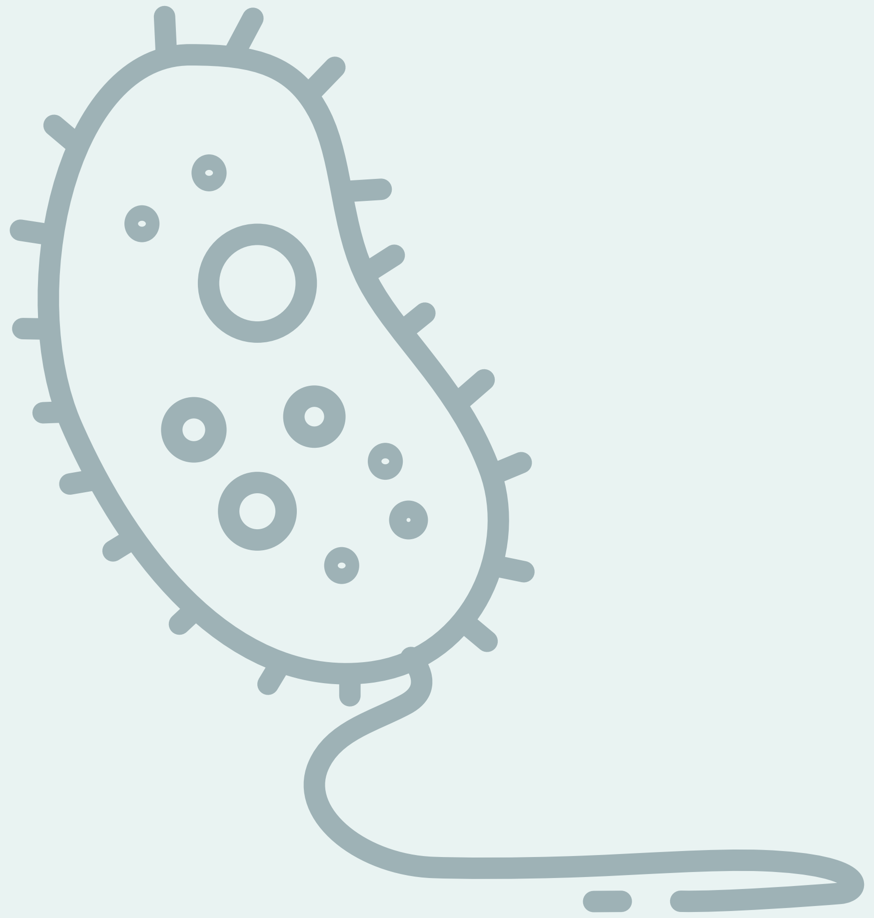
Procedure/Method: During two consecutive breeding seasons we collected fecal samples from black-headed gulls (n=538) and water samples from monitoring locations (n=96) including WWTP:s and downstream aquatic environments including those connected through bird movements. The samples were analyzed using both a culture-based method focusing on Carbapenemase- and ESBL-producing Enterobacterales and a qPCR panel against 48 AMR- and associated genes. We equipped 38 individuals with GPS-tags and analyzed the resulting trajectories using continuous-time movement models.

Findings/Results: We traced Carbapenemase- and ESBL producing bacteria from the WWTP to nesting colonies and gulls, and to areas with potential human impact as recreational sites. While there was great variation both within individuals and population regarding the competency of gulls to act as “AMR vectors”, our results suggest a dispersal pathway via gulls using WWTPs as a food source.

Implications/Applications: Our findings indicate the need to actively implement effective mitigation strategies to reduce the spread of antimicrobial resistance from WWTPs to the environment. Dispersal of AMR from birds feeding in WWTPs must be taken into consideration. Preventing easy access of wild birds to these facilities could be a very feasible first step.

Author: Jonas Bonnedahl, Linköping university, Sweden

Co-Authors: Wennesjö, Pontus; Voksepp, Hanna; van Toor, Mariëlle



Session B

Abstract 202

Poster Board number: 6

Flash Poster Presentation: S18 - Thursday, May 30 - 11:00-12:30

Carbapenemase gene blaOXA-48 detected at six freshwater sites in Northern Ireland discharging onto identified bathing locations.

Background and Aim: This study sought to provide an evidence base for decisions by regulatory agencies if an extension of current surveillance monitoring, of microbial bathing water quality, should include antimicrobial resistant bacteria detection.

Procedure/Method: During the bathing season of 2021, weekly water samples from six selected coastal bathing locations (93n) and their freshwater tributaries (93n), in Northern Ireland (UK), were examined for concentrations of faecal indicator bacteria (FIB) Escherichia coli (E. coli) and intestinal enterococci (I.E). Microbial source tracking (MST) involved detection of genetic markers from the genus Bacteroides using the general AllBac marker, the human HF8 marker and the ruminant BacR marker for the detection of human and ruminant sources of faecal contamination. The presence of beta-lactamase genes blaOXA-48, blaKPC and blaNDM-1 was determined for the investigation of antimicrobial resistance genes (ARGs) that are responsible for lack of efficacy in major broad-spectrum antibiotics. The results of these assays were assessed for statistically significant differences between each location for the sources of faecal bacteria and the presence of antimicrobial resistance genes.

Findings/Results: The beta-lactamase gene blaOXA-48 was found in freshwater tributary samples at all six locations and was detected in 83% of samples that tested positive for the HF8 human marker and 69% of samples that tested positive for the BacR ruminant marker. This study provides evidence for the presence of clinically relevant antimicrobial resistant bacteria (ARB) in tributaries to bathing locations in Northern Ireland. It suggests a risk of human exposure to ARB where bathing waters receive at least episodically substantial transfers from such tributaries.

Implications/Applications: An association of antimicrobial resistance genes with one of the investigated faecal sources would help to prioritise mitigation measures between improvements to sewage infrastructure and management of faecal waste and effluents from agriculture.

Author: Cathy Brooks, AFBI, United Kingdom

Co-Authors: Mitchell, Elaine; Brown, James; Carnaghan, Kelly-Anne; Bleakney, Eoin; Arnscheidt, Joerg

Back to the Program

Session B

Abstract 15

Poster Board number: 7

Comparison of antibiotic resistant bacteria and antibiotic resistance genes abundance in hospital and domestic wastewaters in Brazil

Background and Aim: This study quantifies and characterizes antibiotic-resistant bacteria (ARB) and associated genes (ARGs) in raw wastewater from two hospitals and raw and treated wastewater from two domestic wastewater treatment plants (WWTPs) in Belo Horizonte, Brazil. This work expands knowledge on point sources for ARB/ARGs proliferation and implications for their environmental release in a developing global region.

Procedure/Method: Diluted wastewater samples were introduced to amended agar plates for quantification of ARB resistant to azithromycin, ciprofloxacin, meropenem, and triazole (trimethoprim + sulfamethoxazole) during 3 consecutive weeks. Select colonies were isolated from plates for MALDI-TOF sequencing to identify ARB strains. Enriched colonies were subject to multi-drug resistance assessment for 12 antibiotics, including: amoxicillin, amoxicillin + clavulanic acid, azithromycin, cephalexin, ciprofloxacin, erythromycin, imipenem, meropenem, ertapenem, streptomycin, triazole, and tetracycline. Abundance of 4 ARGs (intI1, sul1, ermB, and qnrB) was over 6 weeks monitored for each sample using DNA extraction followed by qPCR.

Findings/Results: ARB exhibited highest resistance to azithromycin and lowest resistance to triazole and meropenem, with concentrations detected at 5.7±0.7-log in hospital wastewater, 5.3±0.8-log in WWTP influent, and 4.1±1.0-log in WWTP effluent (average 1.2-log removal). MALDI-TOF sequencing revealed presence of 19 bacterial strains, with highest prevalence of E. coli and K. pneumoniae. Antibiogram testing of isolated ARB colonies demonstrated high resistance to triazole, streptomycin, and erythromycin. ARGs were higher in wastewater obtained directly from hospitals than from WWTPs. WWTPs demonstrated an average of 0.85±0.65-log reduction for ARGs during treatment.

Implications/Applications: This study raises concern over antibiotic-resistance levels in wastewater generated from hospitals and other healthcare facilities as point sources in developing regions. It exposes the need for improved discharge management to minimize environmental dissemination of AMR, which increases risks for exposure to economically vulnerable communities. Further, these results illustrate inadequate treatment barriers employed in WWTPs for preventing environmental release of ARB/ARGs.

Author: Juliana Calábria de Araújo, San Diego State University, United States

Co-Author: Astete Vasquez, Lilith; Mladenov, Natalie; Verbyla, Matthew

Session B

Abstract 298

Poster Board number: 8

Household WASH characteristics and ESBL E. coli in stored drinking water: Analysis in Kenya

Background/Aim: Extended-spectrum beta-lactamase-producing (ESBL) E. coli, an indicator of antimicrobial resistance (AR) in the environment, constitute a critical public health threat. This work leveraged ongoing population-based infectious disease surveillance in two diverse settings in Kenya to assess environmental AR in low-resource contexts. We collected environmental samples, which were tested for ESBL E. coli, and data on household water, sanitation, and hygiene (WASH) conditions to identify associations.

Procedure/Method: A cross-sectional assessment of household risk factors and ESBL E. coli in drinking water (DW) was conducted at two sites in Kenya: Kibera and Asembo. The team assessed household WASH conditions using World Health Organization (WHO)/UNICEF Joint Monitoring Programme questions and collected stored DW in the household. Stored DW was tested for free chlorine residual (FCR). If FCR was <0.2 mg/L (WHO recommendation) and the household was among a randomly selected 35% of households surveyed, a 100-mL sample was collected. Samples were tested for E. coli, via IDEXX test using Colilert-18, and for ESBL E. coli, via modified IDEXX test with the addition of cefotaxime. Concentrations are reported in most probable number (MPN).

Findings/Results: From August-November 2023, interviewers collected 696 surveys and 182 stored DW samples in Kibera, and 613 surveys and 201 stored DW samples in Asembo. Preliminary results from Asembo show that 69% (139/201) of lab-tested samples had detectable E. coli and 15% (31/201) had detectable ESBL E. coli. Among samples with detectable E. coli or ESBL E. coli, median concentrations were 12.2 MPN/100 ml (IQR 3.0-57.8) for E. coli and 3.1 MPN/100 ml (IQR 2.0-9.7) for ESBL E. coli.

Implications/Applications: Stored DW, whether contaminated at the source, during transport, or in the household, is a potential exposure pathway to AR bacteria. Further analysis of risk factors will enhance understanding of AR in the environment and inform future ES efforts.

Author: Molly Cantrell, CDC, United States

Co-Authors: Kossik, Alexandra; Ramaswamy, Maya; Odhiambo, Loicer; Oduor, Fredrick; Njugu, Faith; Muchira, Eva; Ndung'u, Mungai; Murphy, Jennifer; Berendes, David; Munywoki, Patrick; Bigogo, Godfrey

Back to the Program

Session B

Abstract 359

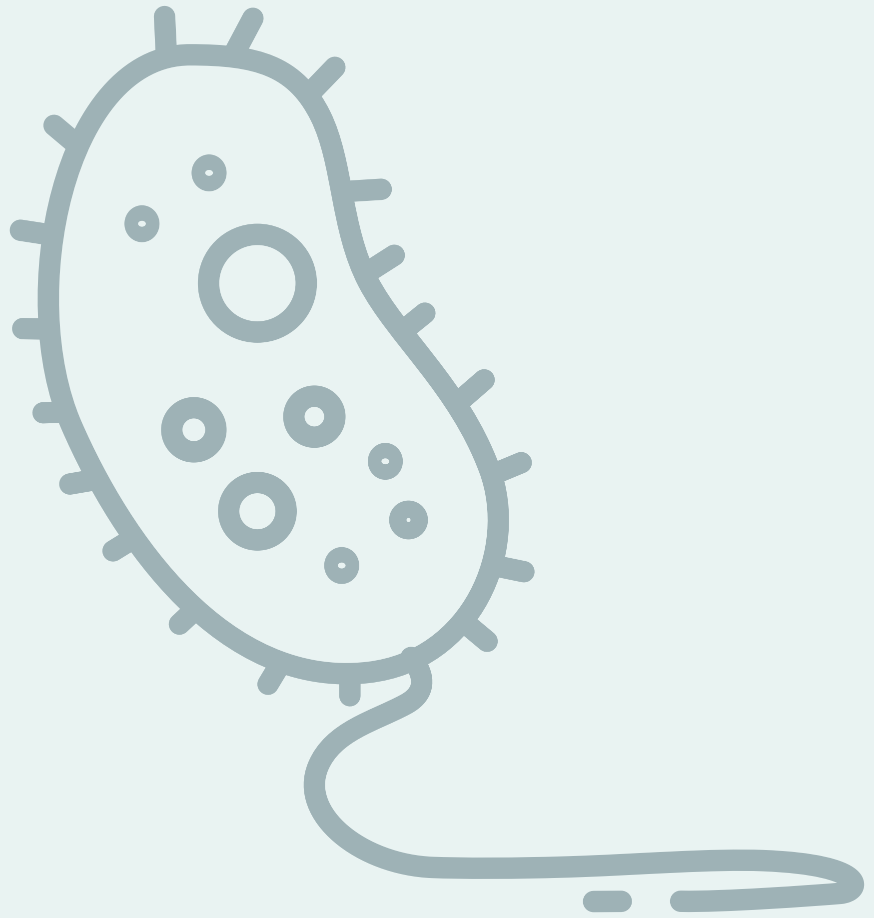
Poster Board number: 9

Antibiotic Resistome in Sewage Pipeline Connected from Hospital Sewage Effluent to a Wastewater Treatment Plant

In a view of recycling of water, pollution of aquatic ecosystem has attracted interest and antibiotic resistance is considered as one of pollutants. In this study, antibiotic resistome was investigated in sequential sewage pipeline connecting to a wastewater treatment plant (WWTP) in a community. Antibiotic resistance genes (ARGs) from shotgun sequencing results were annotated using CARD database and normalized by the number of copies of 16S rRNA gene. The diversity of beta-lactams, aminoglycosides, and MDRs associated antibiotic resistance genes was higher in the WWTP pipeline including influent and effluent before UV treatment, and hospital effluent compared to river samples. ESBL and PABL genes for resistance to third-generation cephalosporin and MBL genes for resistance to carbapenem were observed only in WWTP influent and hospital effluent. This indicates that WWTP and hospital effluent possibly affect the increase of antibiotic resistance in natural environment. In addition, enteric bacteria were isolated using Chromagar Orientation and Chromagar KPC. Pure cultures were identified by MALDI-TOF MS and antimicrobial susceptibilities were analyzed. MLST and ERIC PCR were performed for the clonality of the strains. ESBL-producing E. coli strains carrying CTM-M-14, -15, -27, and -55 genes were mainly detected in the influent of WWTP. Carbapenem-resistant K. pneumoniae (CRKP) ST307 strains were also detected in the influent and harbored SHV-11, CTX-M-15, and KPC-2 genes. The clonal relationships of the CRKP and ESBL-producing E. coli isolates were detected, respectively. This suggested that sewage pipelines could be possible reservoirs for dissemination of antibiotic-resistant clones through water circulation system.

Author: Jong-Chan Chae, Jeonbuk National University, South Korea

Co-Authors: Jeong, Jae Hong; Oh, Jae Young; Kwak, Su Min



Session B

Abstract 113

Poster Board number: 10

Enrichment of antibiotic resistant zoonotic Aeromonas veronii and its potential transmission risk to humans and animals

Background and Aim: Wastewater released from antibiotics-manufacturing facilities can serve as a major source of antibiotic resistome in the environment. However, the variation of antibiotic resistance genes (ARGs) and bacterial communities along the treatment train of antibiotics-manufacturing wastewater treatment plants (AM-WWTPs) and the associated risks to humans and animals are largely unknown.

Procedure/Method: We used deep metagenomic sequencing to systematically analyze ARGs and bacterial communities following the influent to the effluent through the treatment train of eleven units within a full-scale AM-WWTP receiving wastewater from a β-lactams-manufacturing facility.

Findings/Results: The bacterial communities and ARG compositions varied significantly among the treatment units, but were significantly correlated. By assembling metagenomic reads into contigs, certain types of ARGs were carried by the same hosts throughout the treatment units. Some ARGs were physically linked to mobile genetic elements, and hosted by different bacteria, suggesting horizontal gene transfer within the AM-WWTP. More importantly, two metagenome-assembled genomes of zoonotic Aeromonas veronii carrying beta-lactam resistance gene OXA-12, bacitracin resistance gene bacA, and multiple virulence factors were significantly enriched in the effluent. The close phylogenetic relationships between these assembled A. veronii with the isolates from clinic patients and diseased fish found in the local area further confirm that A. veronii originating from AM-WWTP could potentially transmit to humans and animals. The abundance of A. veronii in this AM-WWTP was significantly higher than that found in other non-β-lactams AM-WWTPs, natural waters, and drinking waters worldwide, which is likely attributed to the selection by the manufactured β-lactams.

Implications/Applications: This study reveals a previously unrecognized risk associated with AM-WWTPs, which could contribute to the enrichment and transmission of antibiotic resistant A. veronii, a zoonotic pathogen.

Author: Zeyou Chen, Nankai University, China

Co-Authors: Zhang, Wei; Li, Hui; Tiedje, James. M; Wang, Xingshuo; Zhou, Jizhong

Back to the Program

Session B

Abstract 177

Poster Board number: 11

Flash Poster Presentation: S18 - Thursday, May 30 - 11:00-12:30

Considerations for Wastewater-Based Epidemiology of AMR in Communities with Compromised Sewage Collection Networks

Background and Aim: The COVID-19 pandemic catalyzed widespread interest in wastewater-based epidemiology (WBE), i.e., relating measurements in sewage to carriage of disease agents by surveilled communities. However, to optimize accuracy of WBE models, it is important to consider alterations in signal for health-based targets that occur during sewage conveyance. Infrastructure failures, such as inflow and infiltration (I&I), also make it especially challenging to differentiate precipitation-driven signal changes. The objective of this study was to develop AMR-relevant guidelines for WBE that are adaptive to compromised sewersheds and to test this approach in a low-income community in rural U.S.

Procedure/Method: Sampling was carried out at wastewater treatment plant (WWTP) influent and 12 nodes in the sewershed monthly from September 2022-August 2023. In addition to metagenomic sequencing, ddPCR was used to enumerate intI1, blaCTX-M, and 16S rRNA.

Findings/Results: The highest relative abundances of total ARGs from metagenomic data and highest ddPCR concentrations of intI1, and blaCTX-M-1 were observed at WWTP influent, compared to upstream sites. This is suggestive of selective pressure enriching AMR signal in the sewershed. Further, at WWTP influent, higher abundances of 66 ARGs were also noted on wet weather days, compared to dry weather days (Mann-Whitney U: $p < 0.05$). While no unique ARGs were observed at WWTP influent, 125 ARGs were detected across various upstream sites that were non-detect at influent.

Implications/Applications: Surprisingly, conveyance throughout the sewershed alongside I&I was found to amplify, rather than dilute, total ARGs, an indicator of anthropogenic sources of AMR (intI1), and an ARG of widespread clinical concern (blaCTX-M-1). These results suggest I&I could potentially elevate the diversity of ARGs in sewage through an influx of exogenous ARGs and/or re-suspending ARGs sequestered in biofilms. Contextualizing these findings for other compromised sewersheds can help advance WBE of AMR, addressing specific concerns for under-resourced communities.

Author: Amanda Darling, Virginia Tech, United States

Co-Authors: Deck, Madeline; Byrne, Thomas; Maldonado Rivera, Gabriel; Price, Sarah; Amaral-Torres, Amber; Markham, Clayton; Davis, Benjamin; Vikesland, Peter; Krometis, Leigh-Anne; Cohen, Alasdair; Pruden, Amy

Session B

Abstract 324

Poster Board number: 12

Effects of different wastewater sampling approaches on antibiotic resistance gene determination and other molecular biological analyses

Background/Aim: The widespread use of antibiotics in agriculture, human, and veterinary treatments accelerate the proliferation of antibiotic resistant bacteria (ARB) in the environment. Wastewater treatment plants (WWTPs) serve as hotspot for ARB and antibiotic resistance genes (ARGs) as they can promote ARB proliferation and horizontal transfer of ARGs among microorganisms. Evaluating antimicrobial resistance in WWTPs based on systematically collected data is crucial. However, diverse sampling approaches may impact result accuracy. The aim of this study was to determine how different wastewater sampling approaches affect quantitative ARG analysis and other molecular biological parameters.

Procedure/Method: WWTP influent and effluent samples were collected using different sampling approaches: grab, qualified grab, 1h composite, 4h composite, 24h composite, 7d composite. Additionally, grab samples were stored at 4°C for 14 days to investigate the persistence of different parameters. Samples were filtered and DNA was extracted. The ARG sul1 and the bacterial 16S rRNA gene were quantified by qPCR and DNA quality and quantity was assessed.

Findings/Results: Preliminary results revealed minimal differences in 16S rRNA gene and ARG sul1 concentrations across diverse sampling approaches for effluent and influent. Effluent storage for 14 days at 4°C resulted in a clear decrease in 16S rRNA gene, sul1, and DNA concentrations. Until day 7, no substantial changes in abundance of 16S rRNA gene and sul1 were observed, in line with the results from the different sampling approaches. In the influent, the abundance of the measured parameters remained quite stable even until day 14. Ongoing investigations aim to provide further insights.

Implications/Applications: The preliminary findings suggest that the different sampling approaches that were investigated have limited impact on the quantitative analysis of sul1/ARGs, 16S rRNA gene and DNA, which is in line with the rather stable behavior of these parameters observed in the first 7 days of the storage experiment.

Author: Irina Dielacher, TU Wien, Austria

Co-Authors: Slipko, Katarzyna; Holzwarth, Helene; Woegerbauer, Markus; Galazka, Sonia; Kreuzinger, Norbert; Krampe, Jörg; Vierheilig, Julia

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 199

Poster Board number: 13

Exploration of antibiotic resistome in Hong Kong communities through wastewater metagenomic surveillance

Wastewater surveillance is emerging as an effective tool for disease monitoring. Apart from infectious diseases, antimicrobial resistance (AMR) is recognized as another major threat to global public health. In the present study, we use the metagenomics-based approach to analyze antibiotic resistance genes (ARGs) in wastewater samples from 95 stationary sites in Hong Kong, collected in May 2021 and January 2022, to decipher the population-wide AMR profiles in urban communities. We quantified ARGs to provide their occurrence and distribution information and further identified the relevant antibiotic resistance bacteria (ARBs), especially those ARGs with potentially high risk. Flanking sequence analysis of mobile genetic elements (MGEs) was also conducted based on assembly-based analyses to reveal the potential for gene exchange. Our data gives a representative reflection of the ARG level in Hong Kong communities, which could be useful for public health risk assessment to better combat ARGs and ARBs and thereby protect the health of the public.

Author: Jiahui DING, the University of Hong Kong, Hong Kong

Co-Author: Zhang, Tong

Session B

Abstract 361

Poster Board number: 14

The Australian silver gull plays a role as by-stander host to pathogenic and antimicrobial resistant Escherichia coli

Background: Wild birds carry diverse lineages of multiple-antibiotic resistant Escherichia coli and virulence factors associated with human and avian pathogenesis. Here we describe two extensive whole genome sequencing studies of E. coli sourced from the Australian Silver gull (Chroicocephalus novaehollandiae).

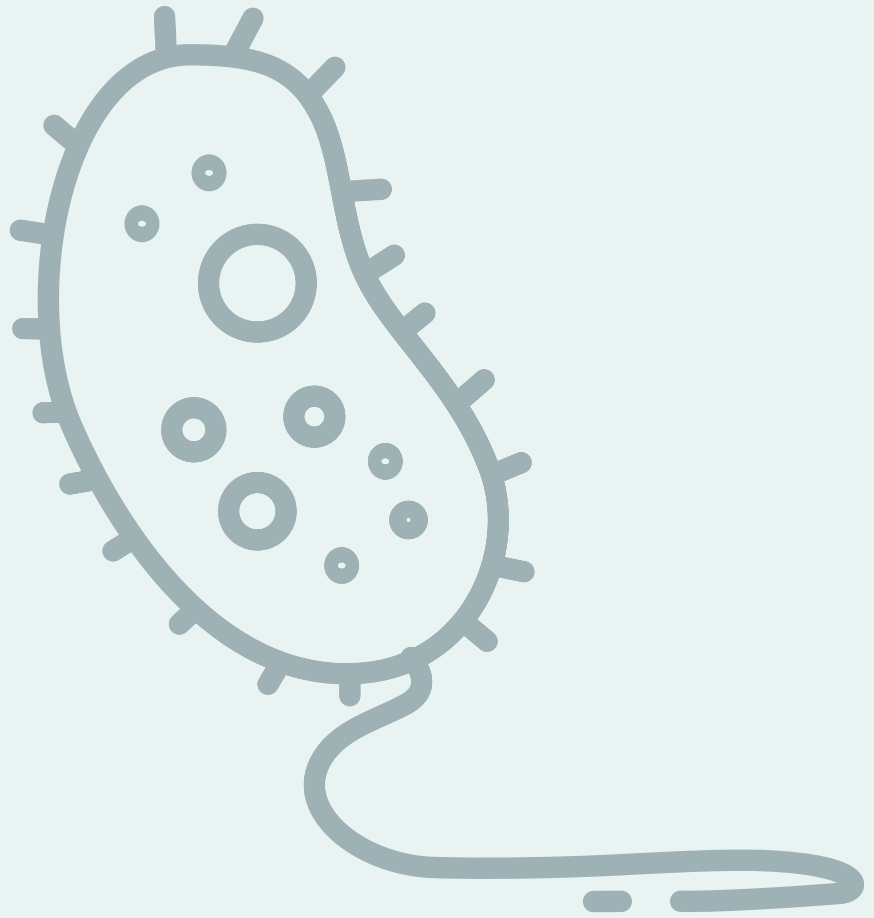
Methods: The first study was comprised of 425 E. coli from cloacal swabs of gull chicks inhabiting three coastal sites in New South Wales, Australia during 2012 that were cultured on selective media supplemented with meropenem, cefotaxime or ciprofloxacin. The second, more recent study, examined 431 E. coli genome sequences from healthy chicks and adults during the 2019 breeding season at one of the three sites, Five Islands, without antibiotic selection.

Results: The 2012 study reported diverse E. coli sequence types (STs) resistance to three or more clinically important antibiotic classes. Further interrogation of the genomes identified the transmissible locus of stress tolerance among multi-drug resistant phylogroup A and B1 lineages. Twenty-five STs hosted carbapenemase gene blaIMP-4, the dominant carbapenemase gene recovered from Australian hospitals. Multiple plasmid types mobilised blaIMP-4 and blaOXA-1, and 121 isolates (28%) carried either a ColV-like (18%) or pUTI89-like (10%) F virulence plasmid. Carriage of the Yersinia High Pathogenicity Island (HPI), a key ExPEC virulence determinant, was a feature of both collections but was more prevalent in the 2019 study, where phylogroup B2 lineages carrying distinct ColV-like plasmids were prominently represented. Clusters of closely related E. coli (<50 SNVs) sourced from healthy gulls, humans, and companion animals were frequently identified, and serve as evidence of interconnection for One Health epidemiological surveillance.

Implications: Our data suggests gulls play an important role in the evolution and transmission of pathogenic and drug resistant E. coli, and caution against studies that ignore virulence gene carriage in lieu of an AMR focus.

Author: Steven Djordjevic, University of Technology Sydney, Australia

Co-Authors: Wyrsch, Ethan; Nesporova, Kristina; Bitar, Ibrahim; Maute, Kimberly; Sanderson-Smith, Martina; Gorman, Jody; Cummins, Max; Jarocki, Veronica; Tarabai, Hassan; Literak, Ivan; Marenda, Marc; Dolejska, Monika; Hoye, Bethany



Back to the Program

Session B

Abstract 228

Poster Board number: 15

Flash Poster Presentation: S18 - Thursday, May 30 - 11:00-12:30

Sewage surveillance of antibiotic resistance - possibilities, limitations and pitfalls

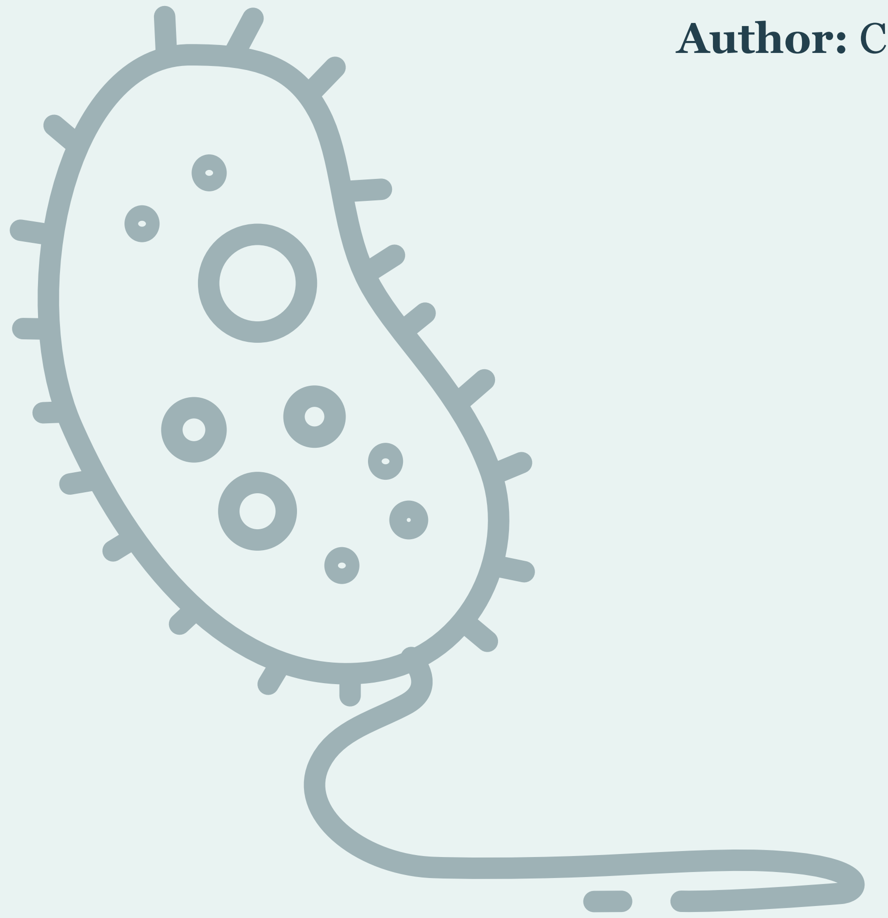
Background and Aim: Surveillance of antibiotic resistant bacteria is a cornerstone for effective management of antibiotic resistance. Since sewage can contain shed bacteria from thousands of individuals, it could provide information about resistant bacteria in the local population. Sewage surveillance is commonly based on either phenotypic analyses of bacterial isolates or on gene-based culture-independent analyses. Each approach has its specific pros and cons as we have outlined recently (https://doi.org/10.1038/s41579-021-00649-x). However, despite promising data and the increased attention given to wastewater-based epidemiology lately, it is still far from clear that it can live up to its suggested potential. The aim of my presentation is to describe and scrutinize validity of different approaches, including sampling strategies, with respect to the different objectives sewage surveillance may have.

Methods: By using both isolate- and gene-based approaches, we have in several recent and ongoing studies evaluated the possibility of using sewage monitoring as a resource-efficient way of predicting the prevalence of ARB in the contributing population as well as identifying outbreaks and novel genes.

Results: Sewage-generated data from these and other studies often show good correlations with resistance rates in clinical isolates, but with important exceptions. The possible reasons for this will be highlighted, some of which can be circumvented more easily than others. The potential of detecting outbreaks with carbapenemase-producing Enterobactereales through either targeted cultivation or gene analyses depends strongly on the species and gene(s). Possible pitfalls potentially leading to misinterpretations will be highlighted. We have also identified completely new, emerging resistance threats in sewage, but methodological complexity makes it currently more suitable for dedicated research efforts than regular surveillance.

Implications: Sewage-based surveillance of antibiotic resistance could be a seminal complement to existing human healthcare systems, not least in regions with limited resources. Still, limitations need to be acknowledged and numerous challenges remain.

Author: Carl-Fredrik Flach, University of Gothenburg, Sweden



Session B

Abstract 175

Poster Board number: 16

Flash Poster Presentation: S4 - Monday, May 27 - 11:00-12:30

Implementing a Surface Water Pilot within a National, One Health Focused Antimicrobial Resistance (AMR) Monitoring System in the United States (U.S.)

Background & Aim: The U.S. Food and Drug Administration-led National Antimicrobial Resistance Monitoring System (NARMS) was initiated in 1996 to track resistance in enteric bacteria. NARMS' current strategic plan emphasizes the expansion of One Health approaches, which led to a collaboration with the U.S. Environmental Protection Agency (EPA) to conduct a pilot environmental AMR monitoring effort focused on surface water.

Procedures/Methods: The interagency effort initially developed and defined standardized methods, involving 1) culture based approaches for isolating antibiotic resistant bacteria (ARB) of interest (E. coli, Enterococcus, and Salmonella), 2) quantitative measures of dozens of antibiotic resistant genes (ARGs), and 3) broader metagenomic sequencing. Subsequent work leveraged existing EPA programs to provide statistically robust studies at both watershed and national scales.

Findings/Results: A 10 month (July-May) sampling of 35 sites (~600 total samples) was completed using an existing EPA water quality monitoring effort within a mixed use watershed in Southeast Ohio. Resistant E. coli, Enterococcus, and Salmonella were cultured from 25-50% of the samples, and nearly 700 presumptive isolates were preserved for whole genome sequencing. DNA was extracted from all samples for metagenomic and targeted gene analysis. The national scale assessment was initiated in 2023 using EPA's National Rivers and Streams Assessment (NRSA) survey (summers of 2023-2024). Approximately 2000 samples will be collected over the two summers to complete the statistical design with over 1000 samples collected during summer of 2023.

Implications/Applications: The watershed study addresses questions of seasonal and interannual dynamics and local-scales processes that alter ARGs and ARBs in the environment and will serve as a basis for designing potential future watershed studies. NRSA provides national scale levels of AMR as a baseline for measuring long term environmental trends, and provides information on which types of aquatic systems, environmental conditions, and landscape factors are associated with higher AMR prevalence.

Author: Jay Garland, Alison Franklin,

Co-Author: Kim Cook, Patrick McDermott

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 276

Poster Board number: 17

AMR-Ecogenomics: Microbial Ecosystems as Drivers of the Antimicrobial Resistome

Background and Aim: Global warming drives the melting of permafrost, which harbors soil bacteria that remained up to now frozen for tens of thousands of years. There is concern that these bacteria introduce novel antimicrobial resistance (AMR) mechanisms in the microbial genetic pool including human and livestock pathogens. One major challenge, however, is the inability to cultivate up to 90% of environmental microbes. Here we present a method combining on-site sampling and WGS referred to as AMR-Ecogenomics.

Procedure/Method: Soil samples from the Eastern Canadian Arctic and controls from Lévis, Québec were inoculated on site using 12 iCHIPs (128-well anodized aluminum plates with gelling agent). Isolated colonies (N=500) were grown in TSB and R2A for 1-5 days and cryopreserved at -70°C. Long-read DNA sequencing was done using Oxford Nanopore GridION, genomes assembled de novo using our in-house NanoLite v1.1 software; taxonomic assignment was done with GTDB toolkit. AMR genes were predicted with RGI v6.0.2 of the Comprehensive Antibiotic Resistance Database.

Findings/Results: AMR genes were detected against major antibiotics including fluoroquinolones, carbapenems and beta-lactams among Arctic and Quebec soil isolates. Janthinobacterium, Flavobacterium and Pseudomonas spp. were the top 3 dominant taxa with AMR genes in Arctic isolates while Pseudomonas, Serratia and Bacillus spp. were from Quebec. Of 5,232 AMR genes, 61 were plasmid-encoded using MOB-Suite. Only 3 were exclusive to Arctic (vanW-vanY), while plasmidic beta-lactamases (B. cereus class A bla, Pedobacter subclass B3 bla) were exclusive to Quebec.

Implications/Applications: Climate change may revive ancient microbes to which our immune systems have been unexposed. These may propagate AMR to present-day pathogens and vice-versa. There is indeed an urgent need for integrative for AMR monitoring, as WHO predicts AMR will be the leading cause of human deaths by 2050. Using the tools discussed herein allows high-throughput surveillance in both ecosystems and populated areas.

Author: Jeff Gauthier, Sima Mohammadi

Co-Author: Hallett, Emily; Potvin, Marianne; Renaud, Valentine; Quang Henri Nguyen, Guillaume; Kukavica-Ibrulj, Irena; Comte, Jérôme; Levesque, Roger C.

Session B

Abstract 414

Poster Board number: 19

Identifying Antimicrobial Resistance Gene Sequence Variants to Track the Dissemination of Antimicrobial Resistance Between Environmental Reservoirs

Background and Aim : To curtail the spread of antimicrobial resistance (AMR), effective regulatory measures must be established. Wastewater treatment plants (WWTPs) and agriculture are known hotspots of AMR, however, the movement of AMR between these environments remains unclear. Effective approaches to address this question must be inexpensive and sensitive. This study aimed to apply multiplex amplicon sequencing of antimicrobial resistance genes (ARG) to identify sequence variants characteristic of municipal wastewater and agricultural sources. Thus, providing important insights to enable the dissemination of AMR to be tracked between reservoirs.

Method: Samples were collected across Canada from 18 WWTPs, and from cattle, swine and chicken manure. High-throughput qPCR of 216 ARGs (targeting 9 antimicrobial classes) was used to quantify selected ARGs. The ARG sequence diversity and microbial community composition based on 16S rRNA gene were determined by amplicon sequencing using the Illumina MiSeq technology.

Results : Analysis of the microbial community composition revealed significant differences between the wastewater and manure samples. While the influent wastewater contained high relative abundances of the genus Acinetobacter and the family Comamonadaceae, manure samples had high abundances of the families Clostridiaceae and Ruminococcaceae. Quantitative PCR revealed significant differences in the abundance of ARGs between wastewater and manure samples. For example, beta-lactam ARGs were in higher abundance in the wastewater, while tetracycline ARGs were more abundant in manure. Multiplexed amplicon sequencing of 32 of the detected genes was utilized to identify sequence variants originating from the different AMR reservoirs.

Implications: ARGs are often shared between different reservoirs of AMR, making it difficult to accurately track the movement of genes and sources of contamination. The results from this study can be used in the future to track the movement of specific ARG sequence variants, which will aid in the development of regulatory measures to prevent the wider dissemination of AMR.

Author: Claire Gibson, McGill University, Canada

Co-Authors: Klimova, Natalia; Hamilton, John Eric; Kraemer, Susanne A.; Salem, Hend; Walsh, David; Frigon, Dominic

Session B

Abstract 222

Poster Board number: 20

AMR Transmission and Nutrition in Infants in Informal Settlements – Behaviour vs Environment

Background and Aim: Studies that simultaneously characterise environmental exposure pathways of antimicrobial resistance (AMR) and health consequences in low-income settings are almost non-existent, especially where environmental and health data have been collected in tandem. To address this gap, informal settlements (slums) in Dhaka were monitored to quantify relationships between environmental AMR exposures, personal nutritional status, and AMR markers in local infants. Our aim was to understand pathways of AMR exposure and health consequences in vulnerable populations.

Methods: Parallel sampling of household water and sewer drains (n=30) and faecal matter from infants (n=74) was performed in two informal settlements (slums) in Dhaka. Microbiomes (16S Illumina), resistomes (HT-qPCR), and water quality analyses were performed, and bioinformatics network analysis was performed on the data. Over 200 nutritional metrics were quantified for individuals and antibiotic use was estimated. Work with the infants and their families was performed with consent by healthcare colleagues from International Centre for Diarrheal Disease Research, Bangladesh.

Results: Infant faecal resistomes were less diverse than those in parallel environmental samples and were dominated by AMR genes (ARGs) associated with local antibiotic use, whereas faecal microbiomes were most influenced by whether the infant was breast fed or not. Overall, infant "gut" resistomes and microbiomes were more impacted by factors related to behaviour than environmental sources, even though ARG diversity was greater in local environments. Co-occurrence analyses suggests that the environment may source ARGs, but behaviour selects what prevails in the gut.

Implications: Our work shows that environmental AMR exposures do not drive ARG type and diversity in the gut of exposed infants, behaviour does. However, recruitment on ARGs from the environment, possibly related to hygiene. The work shows that integrated sampling and analyses are essential in disentangling links between environmental AMR and human health, especially in low-resource settings.

Author: David W Graham, Newcastle University, United Kingdom

Co-Authors: Zealand, Andrew; Islam, Mohammad Aminul; Stewert, Christopher; Li, Zhelun; Jobling, Kelly; Lietz, Georg; Zhu, Yong-Guan; Bunce, Josh; Su, Jian-Qiang

Session B

Abstract 277

Poster Board number: 21

Flash Poster Presentation: S9 - Tuesday, May 28 - 11:00-12:30

Antibiotics and antibiotic resistance genes in an urbanized River

Background and Aim: River water is contaminated by chemical mixture from different sources. Particular concern arises from antibiotics and antibiotic resistant bacteria (ARB), which reach rivers from wastewater treatment plants (WWTPs). Antibiotics and their transformation products together with other contaminants (e.g. biocides, heavy metals) can cause the disappearance and inhibition of microbial species involved in ecosystem functions and promote the Antibiotic Resistance Gene (ARG) spread among natural microbial populations.

Method: The river Danube was sampled (May, October 2023) before the city (near Dunakeszi, close to the bank filtration water supplies), inside Budapest city (immediately downstream from a WWTP), and after the city, downstream another WWTP. Legacy and emerging contaminants (metals, antipyretics, anti-inflammatories, hormones, anticonvulsants, insect repellents, stimulants, disinfectants, antibiotics) were analysed. The bacteriological contamination, including Clostridium perfringens, potential hosts of ARGs, was assessed. Possible estrogenic effects (Yeast Estrogenic Screen, YES), the mobile genetic element intI1 and sulphonamide resistance genes (sul1 and sul2) were also measured.

Findings: A diffuse pharmaceutical residual concentration in all sampling points with a seasonal difference was found. The most polluted point resulted inside the city in line with the YES test results. The highest amounts of pharmaceuticals were detected for antipyretics and anti-inflammatories (up to 50 ng/L) and for the insect repellent DEET (73 ng/L) in October sampling. Ciprofloxacin and sulfamethoxazole were found in all sampling with the highest values in Budapest (18 ng/L and 27 ng/L, respectively), where the highest ARG abundance was detected; intI1 was dominant downstream from Budapest.

Implications: Although soil infiltration is effective in removing pathogenic bacteria, the bacteriological contamination together with different pollutants, including ARGs, has to be taken into account since the river water is used for drinking purposes. The contaminant diffuse presence downstream from WWTPs highlights the need of urgent strategies for improving its efficiency.

Author: Paola Grenni, National Research Council, Water research Institute, Italy

Co-Authors: Rolando, Ludovica; De Carolis, Chiara; Narciso, Alessandra; Patrolecco, Luisa; Rauseo, Jasmin; Spataro, Francesca; Monostory, Katalin; khayer, Bernadett; Toro, Karoloy; Vargha, Marta; Barra Caracciolo, Anna

Back to the Program

Session B

Abstract 374

Poster Board number: 22

Environmental detection of MRSA and VRE in single-patient rooms

Background and Aim: Hospital-acquired infections are life-threatening and often involve highly resistant organisms. Hospital antibiograms estimate the prevalence of antimicrobial resistant organisms, but antibiograms are resource intensive and biased, because they rely on human sampling. Our objective was to determine if built environment sampling around patients (i.e., floor or bedrail sampling) colonized with MRSA or VRE could detect these pathogens.

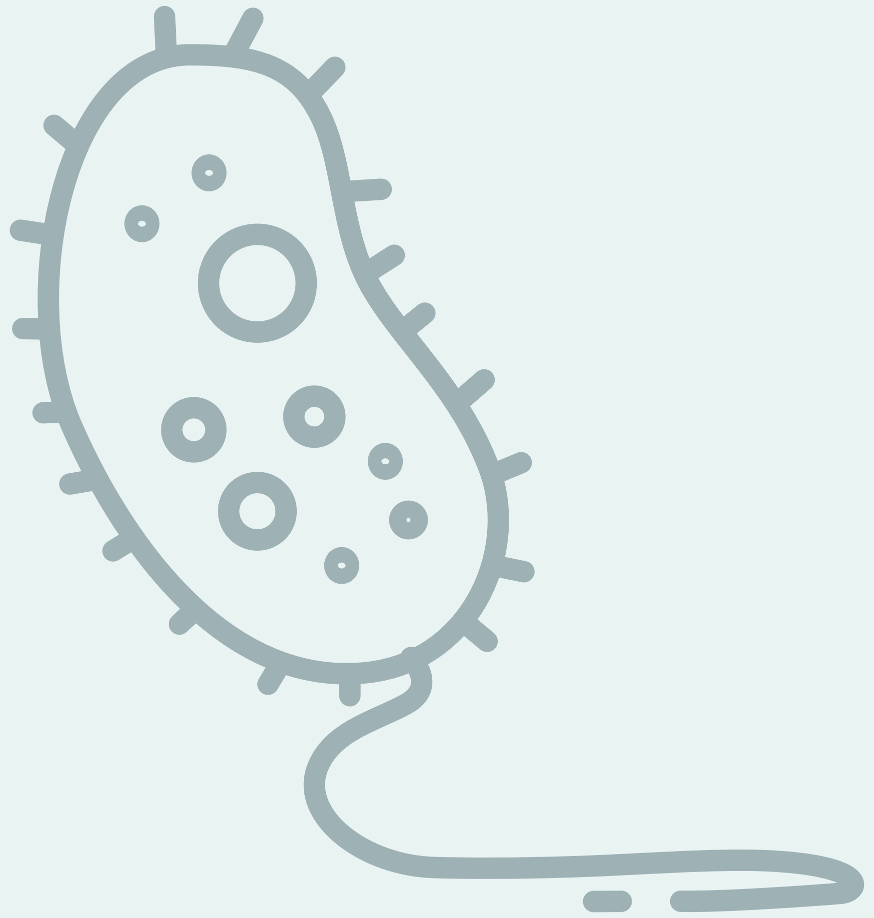
Procedure/Method: Prior studies have shown SARS-CoV2 can be detected in the built environment surrounding infected patients, and these data can be used for early outbreak detection. Using a similar approach, we swabbed floors and bedrails in single patient rooms of patients who were colonized with either MRSA or VRE as confirmed on human sampling. We collected 6 swabs from 4 MRSA and 4 VRE patient rooms, for a total of 48 swabs. From each room, 3 swabs came from the floor and 3 from the bedrails. The liquid from the swab tubes were plated on a panel of selective media, as well as a permissive medium, to identify potential MRSA or VRE. Colonies were isolated from the selective media and frozen in glycerol for further analysis, including minimum inhibitory concentration (MIC) assays.

Findings/Results: We recovered viable MRSA from 1 of 4 MRSA patient rooms, and VRE from 1 of 4 VRE patient rooms. Unexpectedly, we also recovered MRSA from one VRE patient room. In total we collected 7 VRE isolates and 12 MRSA isolates.

Implications/Applications: Further sample collection from single-patient rooms as well as other locations within a hospital would be needed to improve this method of surveillance.”

Author: Alexandra Hicks, University of Ottawa/Carleton University, Canada

Co-Author: Zorcic, Katarina; Ray, Prachi; Hinz, Aaron; MacFadden, Derek; Nott, Caroline; Fralick, Mike; Wong, Alex; Kassen, Rees



Session B

Abstract 154

Poster Board number: 25

Flash Poster Presentation S8 - Tuesday, May 28 - 11:00-12:30

The influence of predation by Tetrahymena borealis on conjugation in Aeromonas salmonicida subsp. salmonicida

Background and aim: The spread of antibiotic resistance genes (ARGs) is driven by horizontal gene transfer (HGT). Several factors can influence the frequency of HGT in bacteria, including other microorganisms present in their environment. Ciliated protozoa may play a role: studies suggest that ciliate predation facilitates HGT in certain bacteria, including Escherichia coli and Aeromonas caviae. To further characterize this phenomenon, A. salmonicida subsp. salmonicida was selected. This fish pathogen carries an extensive and dynamic plasmidome, suggesting a strong potential for HGT.

Procedure/Method: Strains of A. salmonicida carrying a conjugative plasmid bearing ARGs, either pSN254b or pRAS1b, were each cultured with a recipient bacterium, either A. salmonicida, E. coli or A. hydrophila, and with the ciliate Tetrahymena borealis. Conjugation rates were assessed in the presence and absence of T. borealis. Coculture samples were taken for microscopic observation. PCR genotyping was used to confirm the acquisition of the conjugative plasmids and to verify whether other mobilizable plasmids were transferred to the recipients.

Findings/Results: Ciliate predation did not appear to have a significant effect on conjugation rate under the conditions studied, and microscopy observations revealed that most bacteria were digested by T. borealis, leading to a lower conjugation rate. Additionally, it was noted that the basal rate of conjugation in A. salmonicida was quite high. PCR genotyping also showed that some small cryptic mobilizable plasmids found in A. salmonicida were acquired by the recipients during conjugation experiments along with the conjugative plasmids.

Implications/Applications: These results shed light on the ways A. salmonicida plasmids may be dispersed in the environment and show that this bacterium is able to conjugate efficiently with different species. Moreover, our findings suggest that ciliates could mitigate the transfer of ARGs in fish farms.

Author: Alicia Durocher, Université Laval, Canada

Co-Authors: Paquet, Valérie; St-Laurent, Rébecca; Charette, Steve

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 377

Poster Board number: 26

Demonstrating integrated wastewater AMR surveillance for city-scale health protection

Background and Aim: Wastewater-based epidemiology (WBE) has proved to be an invaluable tool for public health protection, exemplified during the COVID-19 pandemic. Pandemic monitoring focused on SAR-CoV-2, but many other genetic, chemical, and biological markers exist in wastewater that can be used for health protection, including for antimicrobial resistance (AMR). Here we quantified antimicrobials, resistomes, and microbiomes from community, hospital, and mixed wastewaters to optimise WBE for AMR surveillance for inclusion in community integrated health planning.

Method: Wastewater samples (n=79) were collected from six in-network community sources (semi-rural, suburban, and urban catchments), a hospital, and a wastewater treatment plant (WWTP) over three months in Newcastle, UK. Analysis was performed on 384 AMR genes (ARGs; high-throughput qPCR) and mobile genetic elements (MGEs), microbiomes (Illumia 16S), over 100 antimicrobials and metabolites (AAs; LC-MS/MS) and AMR bacterial isolates. Wastewater physicochemical parameters were measured and bioinformatics performed.

Results: Wastewater physicochemistry did not significantly differ across sampling sites and no significant differences were observed in total AA, ARG, MGE, taxonomic, and isolate absolute abundances across all sites. However, some ARGs had significantly higher concentrations (gc/mL) in urban locations compared to semi-urban and rural sites. Hospital wastewater contained a one-to-two log higher concentration of ARGs of healthcare concern, quinolones, vancomycin, trimethoprim, beta lactams and aminoglycosides and over 10 times higher concentrations of class 1 and 3 integron cassettes. Significant correlations were seen between related AAs and ARGs in the hospital wastes. Carbapenem resistance-associated genes, such as blaKPC, blaNDM and blaVIM were only found in hospital wastewater.

Implications: Our work shows that WBE can be utilised to monitor AMR at catchment and city scale, link AA used in hospitals and related ARGs in their wastes. The work shows that WBE can identify causes, sources, and types of AMR in different locations to inform public health decisions.

Author: Kelly Jobling, Newcastle University, United Kingdom

Co-Author: Graham, David; Shuttleworth, Joe; Bailey, Olivia; Sims, Natalie; Kasprzyk-Hordern, Barbara; White, Rheanne; Jones, Davey; Osti, Julio

Session B

Abstract 119

Poster Board number: 27

Development of a NIST Standard Reference Material for Environmental and Clinical Antibiotic Resistance

Background and Aim : As the One Health framework for considering antibiotic resistance (AR) gains traction, routine surveillance for AR genes (ARGs) and pathogens is increasing. A necessary element of widespread monitoring is standardized methodology to generate comparable results. This work set out to improve the comparability of ARG assessments by developing a physical NIST standard reference material to enable inter-laboratory comparison via ddPCR and qPCR.

Procedure/Method: Gene targets were identified based on past work by Keenum et al. (2021) as well as through interagency discussions from the EPA, USDA, FDA, CDC and NIST. Gene targets were collated for synergy and the most frequently suggested were selected. One thousand units of material were produced in one day for each unit. The NIST reference material consists of 2, 1mL solutions containing 17 gene targets across the two materials. Each material is provided at two concentrations, 106 gene copies per microliter and 102 gene copies per microliter (for a total of 4 material) and can be stored at four degrees Celsius.

Findings/Results: Lot homogeneity was assessed on each of the 4 materials independently using ten randomly selected tubes and two gene targets measured in technical triplicate. No significant differences were seen in gene copy number based on tube measured or gene target. A consensus concentration was derived for each material by incorporating the variability in gene copy determination across five units for three gene targets. Material stability was assessed over four time points.

Implications/Applications: The production of this material will enable any lab to purchase a reference standard for the evaluation of ARGs in their environment of interest. This will partially synergize assays utilized for ARG monitoring as well as enable labs to confirm the gene copy of their existing standards, or to ultimately replace their standards.

Author: Ishi Keenum, Michigan Technological University, United States

Co-Authors: Stabryla, Lisa; Servetas, Stephanie; Jackson, Scott

Back to the Program

Session B

Abstract 246

Poster Board number: 30

Biodiversity and Antimicrobial Resistance Genes in Bioaerosols: Results of an Across-Canada Assessment using Car Air Filters

Background: Bioaerosols are important but poorly understood vectors for the dispersal of antimicrobial resistant genes (ARGs). These bioaerosols originate from anthropogenic sources, such as agriculture or wastewater treatment, and may contribute to increasing antimicrobial resistance at the landscape level. To assess differences in airborne ARG profiles, we apply a recently developed method using car cabin air filters as proxy air samplers. The goal of this work is to delineate regional ARG profiles and determine links to bacterial communities and anthropogenic activities across Canada.

Procedure: We collected 477 filters from 51 locations. For each filter, population, health care capacity, livestock, and environmental data was collected from publicly available government sources. We developed processing methodology to extract dust from filters for DNA extraction. Copy numbers of 16S and a panel of 39 ARGs were quantified using high-throughput qPCR. Bacterial biodiversity was assessed by 16S amplicon sequencing.

Findings: ARG copy numbers were highest in Ontario and lowest in the Territories. Quinolones were overwhelmingly abundant across all regions. When they were removed, it was revealed that sulfonamide, macrolide, and tetracycline resistance genes had significant, positive relationships with regional livestock populations based on Spearman's correlations. Aminoglycoside resistance genes were positively correlated with human population. Beta-lactam resistance genes were dominant in the Territories, explaining their relationships with temperature. ARG richness was highest in Ontario, the Prairies and the Atlantic provinces. Sequencing data suggest significantly different community compositions between Atlantic Canada and the Prairies and Ontario. For small and rural populations, Shannon-Weiner diversity of ASVs was greater in British Columbia and Prairie samples than in other regions.

Implications: Our findings confirm the viability of car cabin filters as proxy bioaerosol samplers for ARGs and biodiversity data. Results suggest strong links between regional ARG profiles and livestock populations. Results of ongoing network analyses will be presented.

Author: Paul George, Université Laval, Canada

Co-Author: Veillette, Marc; Bélanger Cayouette, Amélia; Leclerc, Samantha; Yao, Maosheng; Turgeon, Nathalie; Duchaine, Caroline

Session B

Abstract 247

Poster Board number: 31

Flash Poster Presentation: S16 - Thursday, May 30 - 8:30-10:15

Monitoring the potential escape of bioaerosolised antimicrobial resistance genes from wastewater treatment plants with air and phyllosphere sampling

Background: Wastewater treatment plants are well known point sources of emissions of antimicrobial resistance genes (ARGs) into the environment. Although most work to date has focused on ARG dispersal via effluent, aerial dispersal in bioaerosols is a poorly understood, but likely important vector for ARG dispersal. Recent evidence suggests that ARG profiles of the conifer needle phyllosphere could be used to measure bioaerosol dispersal from anthropogenic sources. Here we assessed airborne dispersal of ARGs from wastewater treatment plants in Wales, UK and Quebec, Canada, using conifer needles as passive bioaerosol monitors. The aim was to assess the viability of conifer phyllosphere sampling to monitor ARG emissions.

Procedure: Wastewater, air, and conifer phyllosphere samples were collected in Bangor, UK, and Saint-Brigitte-de-Laval, Canada. Samples were collected along a 11 km dispersal gradient in Bangor. Canadian samples were used as a reference point. DNA was extracted following established protocols. ARG profiles of wastewater were compared to those of conifer phyllosphere using high-throughput qPCR based on a panel of 39 genes.

Findings: ARG richness was significantly lower in conifer phyllosphere samples than wastewater samples, though no differences were observed across the dispersal gradients. Mean copy number of ARGs followed a similar trend. Wastewater ARG profiles were similar in both countries, reflecting previous results. However, phyllosphere ARG profiles showed limited, but consistent patterns with increasing distance from wastewater treatment plants, but these did not align with those of wastewater samples. Proportional abundance of aminoglycosides decreased over the dispersal gradient in Wales, whereas mobile genetic elements increased.

Implications: Although ARG profiles were observed along dispersal gradients, links to those of wastewater were not apparent. Agricultural sources of ARGs likely confounded results along the gradient. Longer-term monitoring is needed to assess the viability of conifer phyllosphere as biomonitors of ARG emissions in wastewater treatment plant bioaerosols.

Author: Paul George, Université Laval, Canada

Co-Authors: Hillary, Luke; Leclerc, Samantha; Cooledge, Emily; Lemieux, Joanie; Duchaine, Caroline; Jones, Davey

Session B

Abstract 302

Poster Board number: 32

Flash Poster Presentation: S18 - Thursday, May 30 - 11:00-12:30

Association of population's lifestyles and behaviours with the prevalence of antimicrobial resistance in municipal wastewater

Background and Aim: Wastewater-based epidemiology (WBE) is a promising tool for monitoring AMR in the community. In parallel, few studies have attempted to understand how human behaviors may impact the presence of AMR markers in sewage. The study aimed to identify and quantify the AMR load through the urban water cycle and survey the population to identify critical perceptions and habits that can foster AMR.

Method: 162 samples were collected throughout the urban water cycle, including: source water, drinking water, hospital water inlet & wastewater effluent, and wastewater treatment plant (WWTP) influent, effluent, & waste sludge. A total of 16 points were sampled repeatedly in 3 separate campaigns. Eleven antimicrobial resistance genes (ARGs) were quantified by qPCR (Resistomap). 16S rRNA gene amplicon sequencing was used to describe community compositions. In parallel, a survey aiming to verify perceptions and lifestyle habits that may foster AMR was distributed in selected neighborhoods.

Findings: The widespread presence of ARGs was observed, especially in influent samples, with most ARGs detected in all samples. Prevalence and abundance of ARGs were consistent across WWTPs and seasons; however, a significant increase in abundance was observed between 2020 and the summer of 2022. The detection of broad-spectrum beta-lactamase ARGs, such as NDM, in raw water samples raises environmental concerns despite their absence in treated drinking water. The survey indicated a lack of knowledge towards AMR, leading to behaviors that can promote increased resistance. For example, 12.5% of participants indicated using antibiotics without a prescription.

Implications: Our findings indicate a significant presence of ARGs in our setting, underscoring the value of WBE for monitoring AMR. This research will be instrumental in identifying lifestyle habits and behaviors that can contribute to the spread of AMR, providing decision-makers with evidence to guide policy action and target effective interventions.

Author: Helena Leal, Université De Montréal, Canada

Co-Author: Klimova, Natalia; Gibson, Claire; Fortin, Élise; He, Jie; Frigon, Dominic; Dorner, Sarah; Quach, Caroline; Bédard, Émilie

Session B

Abstract 311

Poster Board number: 33

Barriers and Opportunities for Monitoring AMR in the Environment in LMICs

"Background and Aim: This study begins to address the need for antimicrobial resistance (AMR) monitoring in low-and-middle income countries (LMICs) by building upon work done in the US and Europe. The objectives are to identify barriers to the application of existing methods for AMR monitoring in the context of LMICS, to identify opportunities to improve methods, monitoring systems, or applications in LMICs, and begin the process of incorporating LMICs into Environmental Monitoring programs on an international scale.

Procedure/Method: This project is the first step to establish a baseline around the state of the science on AMR monitoring in environmental matrices in low- and middle- income countries. A comprehensive literature review was conducted using keywords in Web of Science to reduce bias and maximize findings from diverse and varied sources, with each paper reviewed by 2 researchers.

Findings/Results: Key findings include comparisons found in the number of studies conducted in the US and Europe compared with LMICs, resourcing directed through national guidance documents, use of various methodologies, importance of accessibility of materials and resources, and how differences in infrastructure and design impact sampling locations and ease of sampling in LMICs compared with similar monitoring programs in high-income regions.

Implications/Applications: The implications of this research are clear and help to expand the breadth of the work done in the field of environmental monitoring of AMR to a broader audience. This includes regions of the world struggling with active and increasing clinical incidence of AMR infections, with simultaneously less resources allocated to attacking the challenge of AMR from a One-Health perspective. These results have direct applications in building improved international surveillance systems, improving equity and representation in our global datasets, providing data that can be used to inform policy changes in LMICs, and overall helping fight the interconnected global crisis of drug-resistance."

Author: Emilie Gilles, Lehigh University, United States

Co-Author: Liguori, Krista

Back to the Program

Session B

Abstract 14

Poster Board number: 35

Deciphering antibiotic resistome, mobilome, and microbiome of river water in Hong Kong by metagenomic sequencing

Background and Aim: Environmental antibiotic resistance gains increasing attention under the One Health concept. However, it remains underexplored for the resistome and associated mobilome in river ecosystems, and the riverine host of antibiotic resistance genes (ARG) especially the pathogenic host has rarely been studied.

Procedure/Method: In this study, we unveiled the resistome, mobilome, and microbiome of 16 rivers in Hong Kong by combining short-read and long-read metagenomics sequencing.

Findings/Results: Aminoglycoside, bacitracin, β-lactam, macrolide lincosamide-streptogramin (MLS), multidrug, polymyxin, sulfonamide, and tetracycline were the predominant ARG types in the river water. Riverine ARGs exhibited high spatial variations in abundance and diversity in Hong Kong, and the profile was significantly different from other countries or districts. The sources of river ARGs were mainly derived from wastewater treatment plants and sewage. Significant correlations between the ARGs and mobile genetic elements (MGEs) were revealed. The qacEdelta, transposase, integrase, and Tn916 had high prevalence in ARG-carrying long reads. Host tracking using ARG-carrying long reads identified 36 pathogenic bacteria species as the host of ARGs in the rivers. Noticeably, 1 beta_lactam-resistant gene (TEM) and 1 sulfonamide-resistant gene (sul1) were detected as dominant ARGs from pathogenetic hosts and were harbored mainly in the Yersinia frederiksenii_C and Providencia rettgeri_D, respectively.

Implications/Applications: This study demonstrated a nuanced view of antibiotic resistome in river water and the relationship between ARGs with MGEs and pathogens, inflecting the important role of MGEs and hosts in shaping ARGs propagation and distribution.

Author: Xuemei MAO, The University of Hong Kong, China

Co-Author: Zhang, Tong

Session B

Abstract 232

Poster Board number: 37

Flash Poster Presentation: S10 - Tuesday, May 28 - 13:45-15:30

Impact of Wastewater Discharge on Antibiotic Resistance and Microbial Communities in Fish Guts and Urban Rivers

Background and Aim: Wastewater treatment plants (WWTPs) are a major source of antibiotic resistance (AR) to the environment. The objectives of this study were to determine how wastewater effluents impact microbiomes and resistomes of freshwater and fish, and identify potential pathogens carrying clinically relevant AR genes in these matrices.

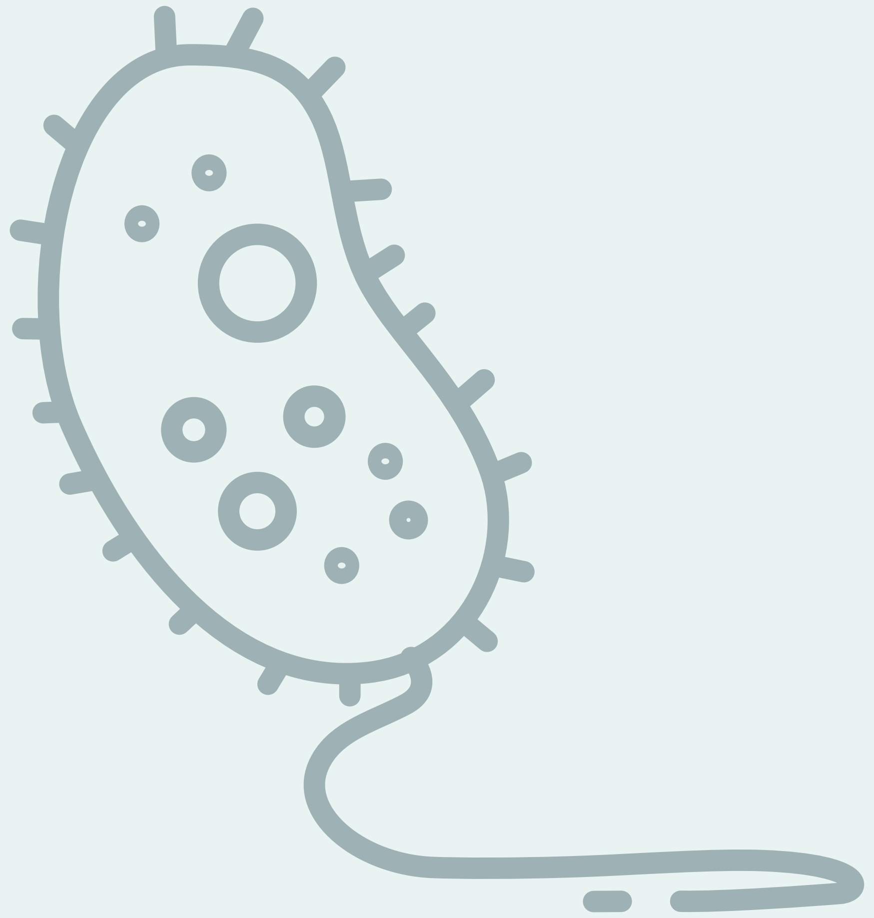
Procedure/Method: We collected wastewater influent and effluent from four urban WWTPs in Ohio, USA. We also collected surface water (SW) and fish from three locations (reaches) in receiving rivers near these WWTPs, including upstream the WWTP outfall, at the outfall, and downstream the outfall. We used shotgun metagenomic sequencing to characterize the microbiomes, resistomes, and microbial AR gene hosts from these samples (n=43).

Findings/Results: The wastewater influent, effluent, water, and fish gut harbored distinct microbiomes and resistomes. There was a trend of decreasing total AR gene abundance from wastewater to receiving SW. The largest number of potentially pathogenic bacteria hosting AR genes were annotated from wastewater influent. However, AR bacteria of public health concern, including potential pathogens hosting carbapenem AR genes, were annotated in treated effluent and SW samples. In SW samples, there were no differences in the microbiome or resistome with reach (upstream, outfall, downstream). There were significant differences in the fish gut microbiome by reach, indicating the sensitivity of this animal gut to ecosystem disruption.

Implications/Applications: In summary, wastewater remains a significant source of AR to the environment. However, urban environments likely have many other sources of AR, so future studies should compare sources of AR pollution. The annotation of potentially pathogenic AR bacteria in treated wastewater and SW indicates that while WWTP processes reduce the hazard from sewage, they do not eliminate all dangerous AR bacteria. Finally, these results highlight the considerable impact of wastewater discharge on aquatic wildlife, which emphasizes the One Health nature of this issue.

Author: Molly Mills, The Ohio State University, United States

Co-Author: Mollenkopf, Dixie; Wittum, Thomas; Sullivan, Mažeika Patricio; Lee, Jiyoung



Back to the Program

Session B

Abstract 189

Poster Board number: 39

Assessing the value of sewage surveillance to predict antibiotic resistance

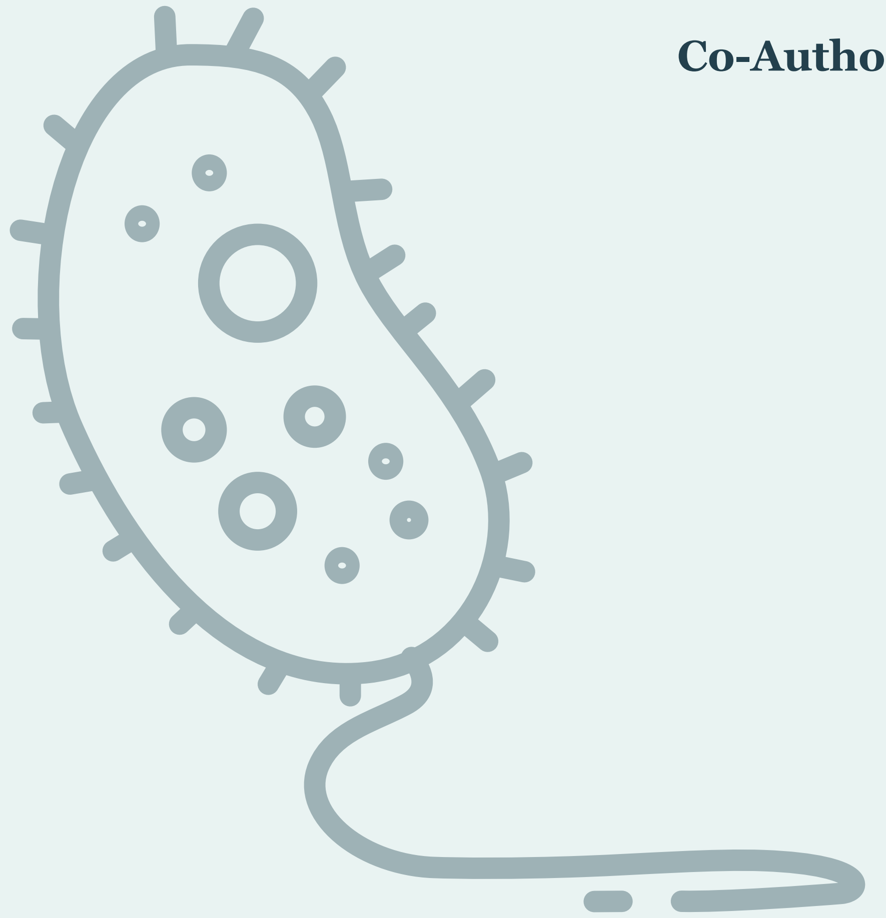
Background and Aim: Antibiotic resistance surveillance data is essential in guiding empirical antibiotic treatment. The underlying data comes primarily from susceptibility tests of clinical samples, which is sparse or absent in most parts of the world due to limited resources. Sewage-based surveillance is proposed as a cost-effective method to predict antibiotic resistance levels in the underlying population. Our overarching aim is to evaluate the economic costs and value of information associated with sewage surveillance compared with clinical point prevalence surveys, to inform empirical treatment in settings with little or no clinical data. Here, we will present the first analyses focussing on the predictive ability of clinical resistance from sewage isolates.

Methods: Culture-based resistance data of Escherichia coli isolates from influents of municipal WWTPs were retrieved from the literature and compared to country-level clinical data using regression analyses. Power analyses will follow to determine optimal wastewater sample sizes, given different resistance scenarios. Preliminary results: Based on 63 study/antibiotic combinations compiled so far, we found a significant relationship between wastewater and clinical prevalence for resistance to aminopenicillins (R2=0.69, p<0.0001), aminoglycosides (R2=0.62, p=0.0038), and fluoroquinolones (R2=0.30, p=0.0297), but not for third-generation cephalosporins (R2=0.03, p=0.5883). We will discuss reasons for sometimes limited correlations, including small datasets, limited range of resistance levels, lack of standardization in both clinical and sewage data, regional differences in the relation between hospital and community resistance prevalence, variable relations between fecal strains and those causing infections etc. An exceptionally strong relationship was found when all antibiotic classes were included in the model (R2= 0.90, p<0.0001), suggesting that approaches that evaluate and incorporate relations in resistance rates between classes might improve precision.

Application: Sewage surveillance data could provide data to guide empirical antibiotic treatment in settings with limited clinical data, but more work is needed to develop optimal approaches for predictions.

Author: Gilbert Osena, University of Gothenburg, Sweden

Co-Authors: Flach, Carl-Fredrik; Kristiansson, Erik; Laxminarayan, Ramanan; Larsson, Joakim



Session B

Abstract 350

Poster Board number: 41

Metagenomic analysis of antimicrobial resistance genes in municipal wastewater in a two-year longitudinal study in Aotearoa New Zealand

Background and Aim: Infectious diseases caused by antimicrobial-resistant microorganisms are a growing global health concern, leading to prolonged illnesses, increased mortality rates, and higher healthcare costs. To combat antimicrobial resistance (AMR), innovative approaches such as wastewater-based epidemiology and metagenomic analysis are needed to complement clinical surveillance. This study focused on understanding the impact of seasons, travel, and COVID-19-related restrictions on AMR in wastewater at the largest municipal wastewater treatment plant (WWTP) in the South Island of Aotearoa New Zealand, serving a population of ~369,000.

Procedure/Method: By analyzing monthly influent sewage samples collected from May 2020 to May 2022 we aimed to identify any correlations between the above mentioned factors and the prevalence of AMR. The sampling started after the first nationwide lock down in March/April 2020 and continued through various levels of COVID-19-related restrictions. During most of the time New Zealand's boarders were closed for international travel. Metagenomic DNA was extracted from monthly samples and deep sequenced (~139 M reads/sample on average, 312 M maximum) on the NovaSeq6000 platform (Illumina), using 2 × 150 bp paired end sequencing.

Findings/Results: Results are presented on a summary level and more detailed for antibiotic resistance genes of concern, such as beta-lactamase genes (including carbapenemase genes), mcr genes, mec genes, and van genes. Preliminary results show that blaNDM genes were detected in 11 out of 23 months, with subtype NDM-8 being the most prevalent. Colistin resistance genes (mcr) were detected in all samples with no seasonal or travel-related correlation apparent. Nineteen of the 26 mcr-genes detected were mcr-3 subtypes.

Implications/Applications: This is the first longitudinal study investigating AMR sewage in Aotearoa New Zealand. Results help to understand the impact of seasons, travel, and COVID-19-related restrictions and give an indication of the prevalence of ARGs of concern over time in the respective population.

Author: Isabelle Pattis, Institute of Environmental Science and Research Limited (ESR), New Zealand

Co-Author: Weaver, Louise; Taylor, William; Dyet, Kristin; McGill, Erin; Sitthirit, Panan

Back to the Program

Session B

Abstract 102

Poster Board number: 42

Flash Poster Presentation: S19 - Thursday, May 30 - 13:45-15:30

Current knowledge of AMR in wild fauna and flora

Background and Aim: Increasing research is being undertaken investigating AMR in wildlife, finding these organisms can act as reservoirs for AMR dissemination and evolution. To identify research trends in AMR hosts and markers for surveillance, a semi-systematic literature review was carried out.

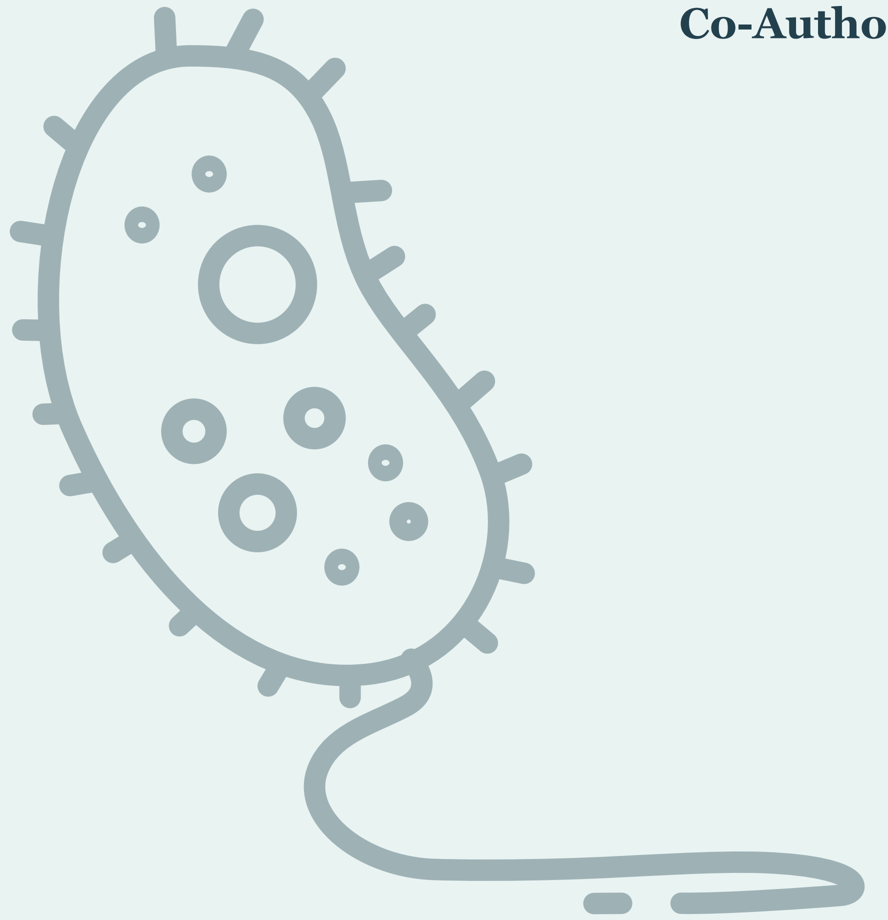
Procedure: Data was extracted from the relevant literature to create a database and metadata was interrogated to investigate trends in the literature. Metadata included: year of study; study location; the species the sample was isolated from; type of sample; microbial and genetic targets; method of AMR analysis; and study rationale.

Findings: A total of 453 publications were included in the final database, published between 1983-2022. Publications increased over time, particularly from 2019. The studies spanned 85 different countries over 7 continents, with the most common being Spain, Portugal, the USA, Brazil and Italy. Mammals and birds were the most studied organisms, followed by other animal taxa, with plants being the least targeted. Sampling types mainly consisted of cloacal/rectal/faecal swabs and faeces. Antibiotic/antifungal susceptibility testing and PCR being the most used methods for AMR analysis, followed by sequencing and whole genome sequencing. Fungal AMR was less well studied in comparison to bacterial AMR and there was a lack of integrated studies examining AMR across multiple environmental niches, such as human, environmental matrices and wildlife (i.e., a “One Health approach).

Implications: Using wildlife for environmental AMR surveillance has the potential to be a powerful tool and can be used by researchers and policymakers to monitor environmental health and provide much-needed data on risks of transmission to human and livestock. This work summarises the research landscape investigating environmental AMR present in wild plants and animals, and gives a broader understanding of its status globally. The knowledge gaps we identified can be used to guide future research priority and funding efforts.

Author: Rachel Payne, UK Centre for Ecology and Hydrology, United Kingdom

Co-Authors: Tipper, Holly; Stanton, Isobel; Shelton, Jennifer; Singer, Andrew; Read, Daniel



Session B

Abstract 403

Poster Board number: 43

Investigating the occurrence of antimicrobial resistance in Canadian environments: A scoping review.

Background and Aim: Antimicrobial resistance (AMR) is an environmental, agricultural, and public health problem that is impacting the health of humans and animals. The role of the environment as a source of and transmission pathway for antimicrobial resistant bacteria (ARB) and antimicrobial resistant genes (ARGs) is a topic of increasing interest that to date has received limited attention. This study aimed to identify the sources and pathways contributing to ARB and ARGs dissemination through bioaerosols (air and dust), water, and soil in Canada.

Procedure/Method: A scoping review methodology and systems thinking approach were used. First, through a literature review process we developed a system map by identifying different bioaerosol, water and soil sources and settings where ARBs and ARGs may be present and potential dissemination pathways between them. The initial system map guided development of the scoping review protocol, specifically the keywords searched, inclusion/exclusion criteria and how and what data were extracted from the included studies.

Findings/Results: In total 103 studies of AMR in water, 67 in soil and 12 in bioaerosols were identified from Canada. Studies to detect the presence of ARGs and ARB have mainly been done in concentrated animal feeding operations (n= 71) and at wastewater treatment plants (n= 51). We also identified several research gaps related to the study of communities, hospitals, aquaculture, and natural environments.

Implications/Applications: The data extracted from these studies will be used to parameterize environmental transmission models for AMR. Specifically, additional pathways will be added to an existing integrated assessment model that investigates the relative human exposure to AMR through different animal production chains. The results of this study and future modelling efforts will provide evidence for public health policy implementation to help minimize exposure to AMR through the environment and identify key environmental settings for intervention.

Author: Manuel Perez Maldonado, University of Guelph, Canada

Co-Author: Ofori-Darko, Daniel; Nichols, Vanessa; Spence, Kelsey; Parmley, Jane; Reid-Smith, Richard

Back to the Program

Session B

Abstract 236

Poster Board number: 44

Comparing the Occurrence of Antibiotic Resistance Genes in Septage and Wastewater Influent in Southwest Virginia

Background and Aim: In the United States, 10-20% of homes are reliant on septic systems for onsite wastewater disposal. Septic system maintenance and treatment efficacy falls outside standard wastewater treatment regulatory frameworks, and risks associated with the contamination of local surface- and groundwaters are often undetected. Extended residence times in settling tanks creates a unique microbial ecosystem, including high concentrations of antimicrobials and other contaminants that could pose selective pressure for antimicrobial resistance (AMR) prior to discharge to the environment via drainfields. The aim of this study was to quantify and compare the occurrence of a suite of antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) within septage and untreated domestic sewage located in the same region.

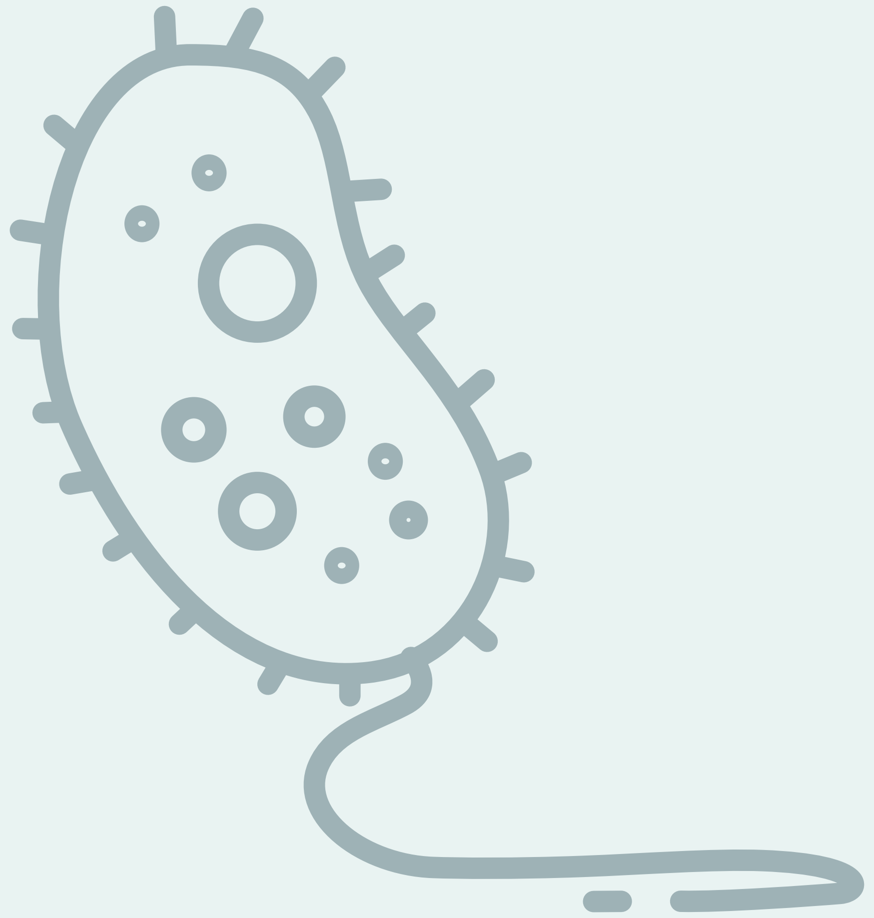
Procedure/Method: Septage (n=52) and influent (n=26) samples were collected twice monthly over the course of one year from a ~9MGD wastewater treatment plant that treats domestic sewage as well as septage collected from systems in seven surrounding counties. A suite of ARGs/MGEs (intI1, sul1, tetA, and blaCTX-M) and 16S rRNA were quantified via digital droplet PCR. Ten months of sampling have been completed, with final sampling anticipated in February, 2024.

Findings/Results: All gene targets were consistently recovered in both septage and influent wastewater. Preliminary trends indicate a higher variance in quantities of the target genes in septage than in influent wastewater, with lower median and average absolute and normalized concentrations in septage.

Implications/Applications: Septic systems with onsite subsurface drainfields are a potentially overlooked nonpoint source of AMR. Ongoing work aims to pair septage manifest and wastewater influent data to examine the potential utility of septage monitoring as a means to extend wastewater-based surveillance to rural populations that are not served by centralized wastewater treatment plants.

Author: Sarah Price, Virginia Tech, United States

Co-Authors: Krometis, Leigh-Anne; Magee, Jessica; Darling, Amanda; Pruden, Amy



Session B

Abstract 164

Poster Board number: 45

Flash Poster Presentation: S9 - Tuesday, May 28 - 11:00-12:30

Hydrodynamic model-assisted tracking of antimicrobial resistance distribution in Lake Geneva

Background: Lake Geneva (Switzerland) receives effluent from the Lausanne sewage treatment plant (STEP) and occasionally untreated bypass. The lake serves recreational purposes and is a source of potable water. Understanding the AMR dynamics in the Lake is useful to determine the AMR distribution and risk associated with wastewater plumes in 3D.

Method: Sampling efforts were guided by modeling the expected movement of the wastewater plume with Lagrangian particle tracking in a hydrodynamic model of Lake Geneva. Water samples were collected at various distances from the wastewater outfall within Lake Geneva and different depths and of STEP effluent. Filtration for DNA extraction, followed by quantification of 16S and ARGs (intI1, sul1, sul2, tetW, tetM, qnrA, ermB, blaCTX-M-1, vanA), was conducted. 16S amplicon and shotgun metagenomic sequencing were performed with a GridION Oxford Nanopore Sequencer.

Results: The ongoing study confirmed the presence of AMR indicators and clinically relevant ARGs (intI1, sul1, sul2, tetW, tetM, ermB, and blaCTX-M-1) in the STEP effluent, and within the Lake. ARGs were at least two-log higher in the STEP effluent and half-log higher at Lausanne Beach compared to the Lake samples. We observed increased abundance of sul2, tetW, and tetM at the STEP outfall, and transport along the model-predicted path at or below the thermocline (25 and 30.5 m) compared to locations outside of the predicted plume or in surface water (2 m). The presence of wastewater bacteria (Thermomonas, Aeromonas, Dechloromonas, Ferruginibacter, Zoogloea, Rhodobacter, etc.) further supports influence of the wastewater plume. These results substantiate the correctness of the predicted plume.

Implications: The results provide crucial insights into the role of wastewater transport on AMR prevalence and 3D distribution within Lakes, and a basis for incorporating AMR fate and risk assessment into lake water quality models.

Author: Sasikaladevi Rathinavelu, Eawag, Switzerland

Co-Author: Beck, Karin; Bouffard, Damien; Irani Rahaghi, Abolfazl; Buergmann, Helmut

Session B

Abstract 122

Poster Board number: 49

Flash Poster Presentation: S17 - Thursday, May 30 - 11:00-12:30

Impacts of Antibiotic Pesticide Use in Crop Agriculture on Antibiotic Resistance in Human Pathogens and Commensals: A Systematic Review

Background and Aim: There is concern that the use of antibiotics as pesticides in crop agriculture may contribute to the emergence and maintenance of antibiotic resistant organisms and/or increase the risk of antibiotic resistant infections for people in contact with treated crops or the surrounding environment. The aim of this work was to synthesize available evidence on the effects of the use of antibiotics as pesticides on the development of antibiotic resistance in human commensal bacteria or pathogens.

Method: A systematic review was conducted following the PRISMA guidelines to capture primary evidence related to the impact of antibiotic pesticide use on antibiotic resistance outcomes such as relative abundance of resistant bacteria, resistance genes, and mobile genetic elements.

Findings: The systematic review identified 13 primary research articles. We identified four study designs from reviewed studies that directly assess the effect of antibiotics used as pesticides on the development of antibiotic resistance in human commensal bacteria or pathogens. These studies provide evidence of transient selection of resistant pathogens and commensals in the plant phyllosphere following the application of antibiotics at agricultural rates. There remains a lack of information on: the types of organisms with resistance genes; the potential role of mobile genetic elements associated with resistance genes; possible impacts on the emergence of new forms of resistance; the duration of resistance effects over multiple seasons or multiple applications; and quantification of absolute abundance of resistant pathogens or commensals and ARGs in relevant matrices.

Implications: The majority of the reviewed research was not designed to inform impacts on human health and it remains unclear if these effects lead to resistant infections in humans or the emergence of new forms of resistance. We recommend a set of minimum requirements for studies to evaluate an effect of antibiotics on resistance in crop agriculture.

Author: Mary Schoen, Soller Environmental, United States

Co-Authors: Jahne, Michael; Garland, Jay

Session B

Abstract 310

Poster Board number: 50

Flash Poster Presentation: S11 - Tuesday, May 28 - 13:45-15:30

Evaluating a metagenomic workflow for nontargeted detection and quantification of AMR and disease agents in wastewater

Background/Aim: In the wake of the COVID-19 pandemic, there is interest in expanding wastewater-based surveillance (WBS) infrastructure to capture multiple pathogens and AMR indicators. Metagenomic sequencing holds promise for achieving this aim as a nontargeted approach that can capture a broad swath of pathogens and antibiotic resistance (ARGs). Here we carried out a controlled spike-in experiment to establish detection limits, quantitative capacity, and ability to distinguish different strains using metagenomics.

Procedure/Method: A cocktail of inactivated bacterial, viral, protozoal, and fungal pathogens and three ARGs was spiked into the influent and primary solids of two wastewater treatment plants (WWTPs) and subject to two filtration workflows or direct extraction, respectively. Three extraction kits optimized for DNA, RNA, and total nucleic acids (TNA) were compared to assess the relative recoveries of each target via digital droplet PCR (ddPCR). Extracts were spiked with quantitative internal reference ladders prior to deep Illumina sequencing (120 Gb). Metagenomic data was benchmarked to ddPCR data to evaluate the accuracy and sensitivity.

Findings/Results: The TNA extraction kit provided similar or improved recovery of all targets compared to DNA- and RNA-specific kits. Deep shotgun sequencing of wastewater DNA extracts resulted in an estimated limit of detection (LOD) of 83 gc per µL extract. Concentrations of four ARGs present above this LOD were congruent between metagenomics and ddPCR (percent error versus ddPCR < 40% for all samples). For blaOXA and blaCTX-M, metagenomic analysis was also able to distinguish between the fraction of ddPCR-amplifiable and non-amplifiable alleles, demonstrating that metagenomics can provide accurate and unbiased quantification of a broader range of gene variants than ddPCR.

Implications/Applications: This study demonstrates capacity of metagenomics for quantitative WBS of multiple public health threats. With TNA extraction, workflows for ARG and pathogen surveillance can potentially be harmonized.

Author: Katherine Scott, Virginia Tech, United States

Co-Author: Brown, Connor; Song, Haoqiu; Blair, Matthew; Markham, Clayton; Davis, Benjamin; Zhang, Liqing; Vikesland, Peter; Pruden, Amy

Session B

Abstract 303

Poster Board number: 51

Bioaerosols in Arid Climates: A One Health Approach to Antibiotic Resistance and Viral Surveillance in Qata

Background and Aim: This study marks the first comprehensive investigation of ambient air bioaerosols in Qatar, focusing on the environmental dimensions of antibiotic resistance and viral diversity. It aims to understand the public health implications of airborne superbugs and viruses in an arid climate, particularly in urban areas of Doha.

Procedure/Method: Bioaerosol samples were collected from eight different urban sites in Qatar in six cycles, encompassing varying climatic conditions. Advanced methodologies, including classical microbiology and next-generation sequencing, were employed to analyze these samples for a broad spectrum of pathogens, including bacteria, fungi, and viruses.

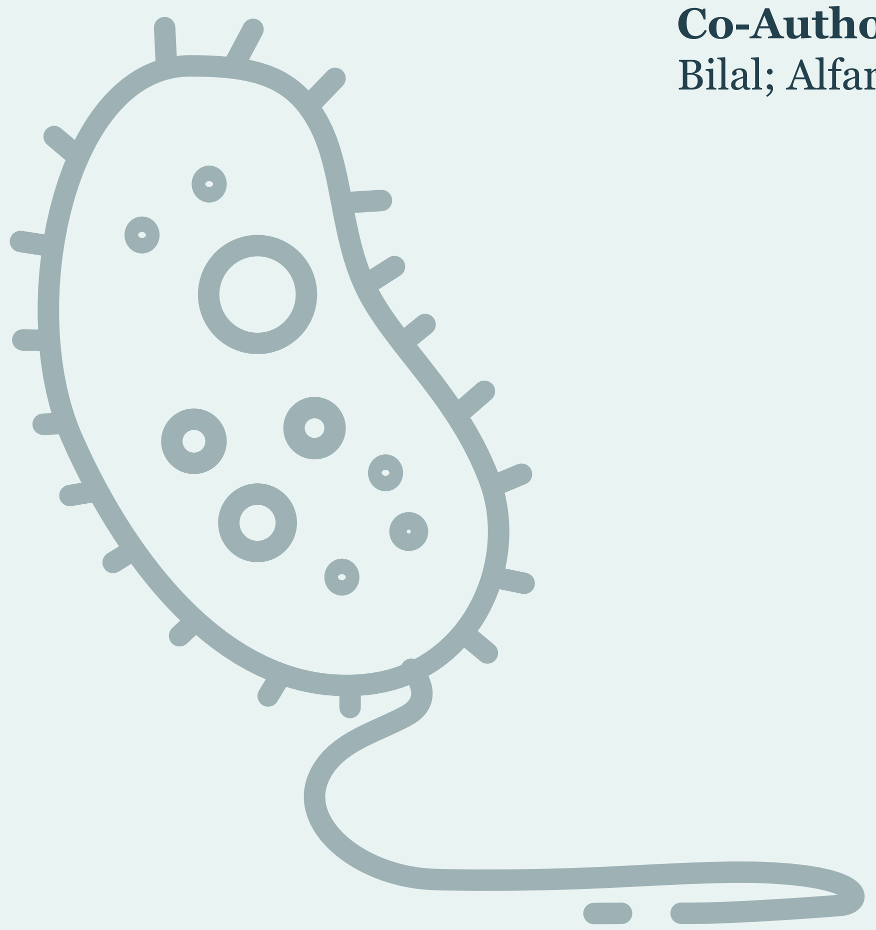
Findings/Results: The study revealed significant findings in two key areas:

1. Antibiotic Resistance Genes (ARGs): The presence of critical and high-priority antibiotic-resistant strains, such as Acinetobacter baumannii and Staphylococcus aureus, was identified. Spatial and temporal differences in ARG profiles indicated a notable public health concern for urban air quality.
2. Viral Diversity: A wide array of viruses, including zoonotic and plant viruses, was detected. Noteworthy are human pathogens like Escherichia coli and Orthohepevirus A (Hepatitis E virus), underscoring the potential for airborne transmission of diseases.

Implications/Applications: This study highlights the intricate relationships between human, animal, and environmental health in urban ecosystems. The findings emphasize the importance of integrated surveillance for a comprehensive understanding of bioaerosol composition and the potential public health risks they pose. This is particularly pertinent in the context of Qatar's unique climatic conditions, where dust storms can significantly influence bioaerosol dynamics. The study advocates for heightened awareness and proactive public health measures to mitigate the risks associated with airborne pathogens and antibiotic resistance.

Author: Azhar Siddique, Qatar Environment and Energy Research Institute, Hamad Bin Khalifa University, Qatar

Co-Authors: Rasool, Kashif; Elmallah, Shaimaa Samir E. Ali; Abdul Jabbar, Khadeeja; Sajjad, Bilal; Alfarra, Mohammed Rami



Session B

Abstract 23

Poster Board number: 52

Flash Poster Presentation: S7 - Tuesday, May 28 - 8:30-10:21

Antibiotic Resistance Genes in Wastewater and Environmental Water and Implications for Thailand National Action Plan on Antimicrobial Resistance

Thailand initiated the first phase of the National Action Plan on Antimicrobial Resistance from 2017-2021. To further this initiative and develop effective strategies, Thailand issued the 2022 Integrated Antimicrobial Resistance Surveillance with One Health Approach Guideline. This emphasizes gathering data on antibiotic resistant bacteria and antibiotic resistance gene (ARG) distributions across diverse environmental matrices and throughout different seasons. This study contributes to the Framework by assessing the levels and distribution of ARGs in untreated and treated wastewater, as well as river water, during dry and wet seasons. High-throughput quantitative PCR analysis of 46 ARGs was conducted and normalized to the 16S rRNA gene, providing relative abundance. Eight selected genes were quantified using qPCR analysis to provide absolute concentrations. The study reveals a substantial and largely uniform presence of ARGs across all water sample types. Notably, no ARGs were exclusive to specific water types, indicating extensive circulation of resistance determinants across aquatic environments. High relative abundance of ARGs across all water types included qepA, aadA2, merA, sul1, along with mobile genetic elements like intI1. Notably, there was no apparent spatial distribution pattern among upstream and downstream rivers nor differences among the three WWTPs. Moreover, no temporal distribution among the four sampling events was observed. This could indicate the consistent presence of these genes, irrespective of the season. The study also found a significant reduction of all genes throughout the wastewater treatment processes but noted their persistence in effluent wastewater. This indicates that current wastewater treatment methods may not be effective enough. The high correlation between crAssphage and each of the seven ARGs demonstrated its potential utility as a human-specific fecal indicator in sewage. These findings underscore the need for efficient measures to reduce antibiotic resistance dissemination in water systems and highlight the role of effective treatment processes.

Author: Kwanrawee Sirikanchana, Chulabhorn Research Institute , Thailand

Co-Author: Srathongneam, Thitima; Sresung, Montakarn; Paisantham, Phongsawat; Raksakul, Pacharaporn; Honda, Ryo; Singer, Andrew; Sukchawalit, Rojana; Satayavivad, Jutamaad; Mongkolsuk, Skorn

Back to the Program

Session B

Abstract 365

Poster Board number: 53

Small river meets big city: Sewage pollution drives AMR and detectable through simple proxies

Background and Aim: Globally, India ranks third largest producer of pharmaceuticals and highest consumer of antibiotics. Within India, Hyderabad accounts for 40% of the total pharma production including 182 drug manufacturers whose effluents are diverted to Amberpet STP for a combined sewage treatment operation and finally discharged into River Musi. About 770,000 m3/day of treated and 940,000 m3/day of raw sewage are estimated to be discharged into the Musi. This study aims to track antimicrobial resistance (AMR) conditions in a highly sewage impacted river catchment and identify simple markers that mirror elevated AMR due to anthropogenic activities.

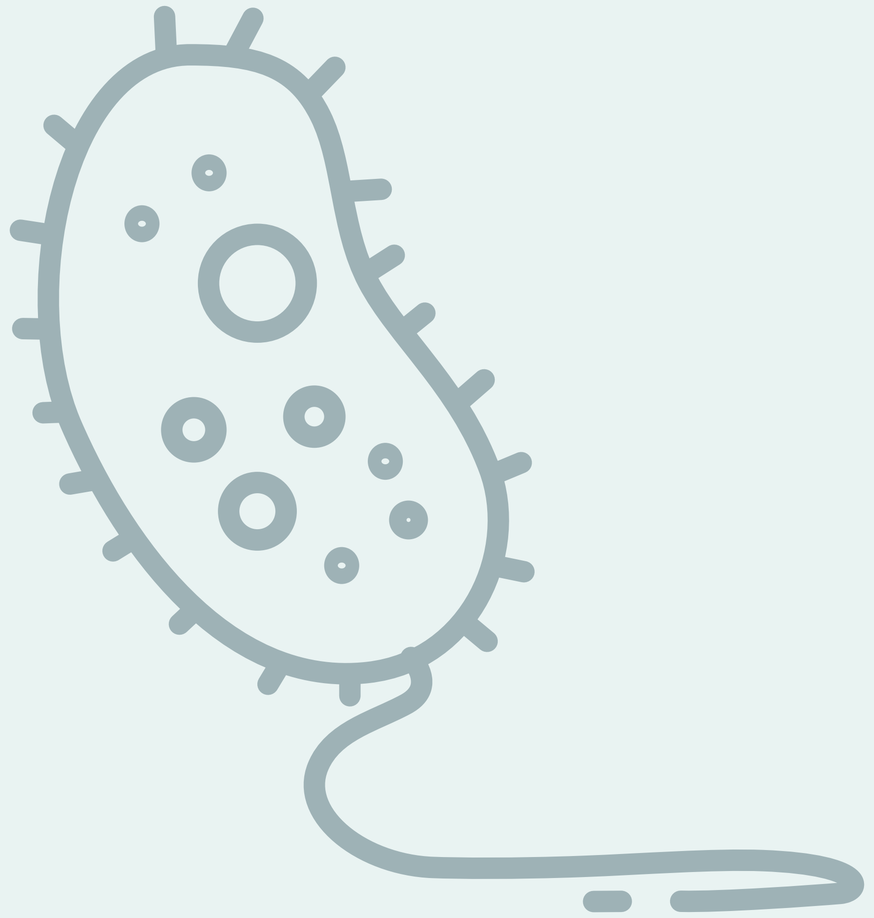
Procedure: We performed a comprehensive spatio-temporal assessment of river Musi. Ten sites (Water and sediment samples) along a river stretch of 153 Km were sampled during dry and wet seasons. Environmental parameters of collected samples were analyzed and the prevalence of 16S, E. coli marker gene uidA, intI1 and seven AMR genes were measured by qPCR.

Findings: Hierarchical clustering of resistance genes specifically aph(3)-lb, ermF, sul2, qnrS and tetW, and MGE intI1 clearly distinguished three river stretches during dry season: upstream; within the city; and downstream of the city. During wet season, the increase in resistance within the city was more gradual and the clusters only partially corresponded to the stretches. Linear Discriminant Analysis identified environmental variables that could distinguish the three river stretches. DO (low within the city) and Total Nitrogen (TN) (high within the city) were sufficient to distinguish the river stretches.

Implication: The elevated levels of 16S rRNA, AMR and TN and the decline in oxygen in the city stretch can be explained by the substantial influx of untreated sewage. Thus, we propose using DO and TN measurements as an alternative to qPCR in resource-limited settings, serving as proxies for sewage pollution and, consequently, AMR pollution.

Author: Vikas Sonkar, IIT Hyderabad, India

Co-Authors: Kashyap, Arun; Pallarés-Vega, Rebeca; CJ, Sangeetha; Kreft, Jan-Ulrich; W. Graham, David; T, Shashidhar



Session B

Abstract 72

Poster Board number: 54

Differential Antibiotic Resistance Profiles in Wastewater and Public Spaces Across Varied Housing Densities in Makassar, Indonesia

Makassar, the fourth largest city in Indonesia in the southern part of Sulawesi Island, is a pivotal hub for the eastern region of Indonesia. The city's socioeconomic landscape exhibits a stark imbalance, characterized by pockets of low-density, affluent housing juxtaposed with high-density slum areas. Domestic wastewater treatment is decentralized, relying on septic tanks within individual households. This study focused on isolating Escherichia coli (E. coli) from wastewater in drains (n = 80) across areas with diverse housing densities, ranging from high to low density. Additionally, E. coli samples were collected from various surfaces (toilet seat, water tap, and carpet, n = 68) within mosques in each area to explore antibiotic resistance profiles within these distinct populations. The susceptibility of the isolates was assessed using the Kirby-Bauer disc diffusion test against amoxicillin-clavulanate, amikacin, chloramphenicol, norfloxacin, and trimethoprim. Our findings revealed a significantly higher proportion of resistant isolates (70%) in high-density areas, with 30% exhibiting multidrug resistance, compared to low-density enclaves (30% resistance, 5% multidrug resistance). Notably, resistance to all tested antibiotics was prevalent in high-density areas, while isolates from low-density enclaves demonstrated resistance only to amoxicillin-clavulanate and trimethoprim. Contrasting results emerged from the mosque samples. High-density enclaves exhibited two resistant isolates, whereas low-density enclaves displayed four. Isolates from low-density areas demonstrated resistance to amoxicillin-clavulanate, amikacin, and trimethoprim, while those from high-density areas were resistant only to amikacin. This discrepancy suggests that high-density housing and inadequate wastewater infrastructure may contribute to the proliferation of antibiotic-resistant bacteria. Importantly, the resistance profiles in wastewater did not align with those observed in the mosque samples, emphasizing the need for distinct sampling approaches when monitoring antibiotic-resistant bacteria in populations similar to Makassar's context.

Author: Sulfikar Sulfikar, Universitas Negeri Makassar, Indonesia

Co-Authors: Herman, Rismawati; Ruslan, Zuhrah Adminira; Afdal, Muhammad; Saputra, Akmal

Back to the Program

Session B

Abstract 229

Poster Board number: 55

GastroPak : Tracking sources of gastroenteritis in Pakistan

Background and Aim: Gastroenteritis, especially that caused by antibiotic resistant pathogens, is a serious health problem in many low- and middle-income countries, causing over a million deaths a year and effecting long-term health impacts in the most vulnerable. Gastrointestinal pathogens are found not only in humans, but also in animals, crops, and the environment. These sites interact in complex ways, and each can act as a source of infections for the others, meaning to tackle gastroenteritis we need a "One Health" approach. GastroPak is a multi-national, cross-disciplinary project tracking non-viral sources of gastroenteritis in Pakistan, developing interventions to reduce infections, and building resource capacity in Pakistan.

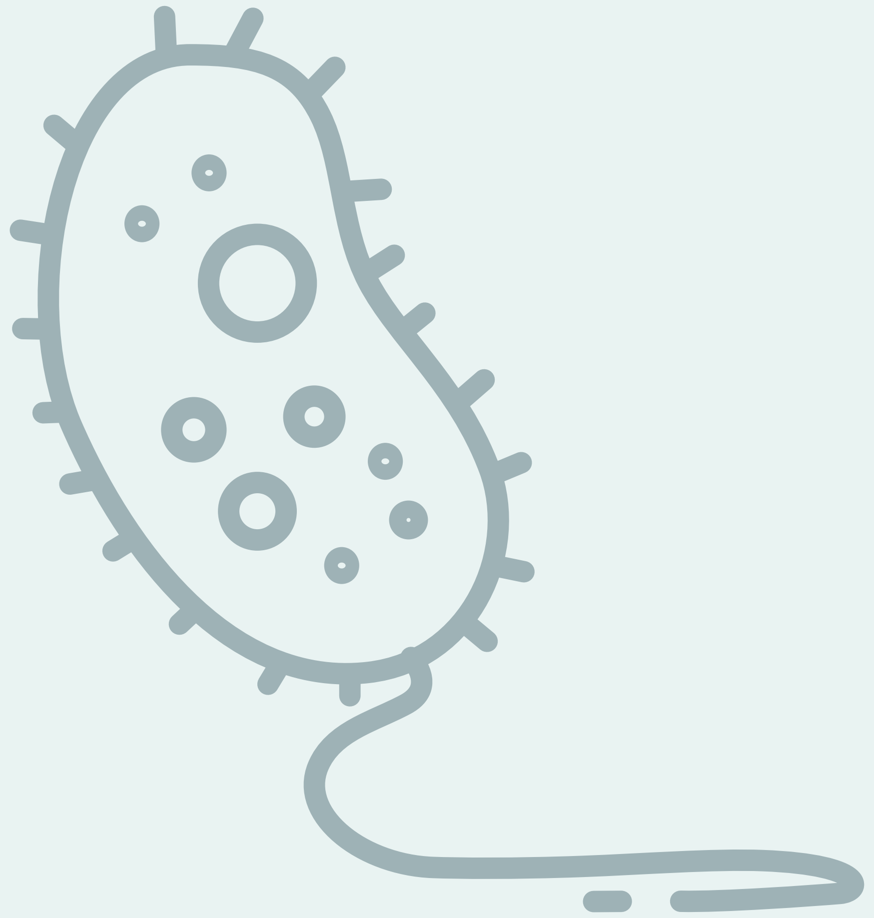
Procedure/Method: We are combining social science, epidemiology, soil science, selective culture, and molecular microbiology to develop a comprehensive understanding of infection routes and antibiotic resistance profiles and design meaningful and achievable interventions.

Findings/Results: Our sampling of five key rivers in the Islamabad area showed an increase in antibiotic resistance genes in downstream regions, compared to upstream and particularly high levels of aminoglycosides and sulfonamides. Concurrently our household surveys in the same area revealed faecal contamination of drinking water and the presence of Campylobacter spp. in the gut communities of residents.

Implications/Applications: Identification of sources of bacterial gastric pathogens is allowing us to develop specific, targeted interventions, such as a boil water advisory for households where faecal bacteria were detected in the drinking water supply. By working with social scientists and local communities and basing interventions on scientific evidence we can increase the likelihood of compliance and reduce the risk of disease. Simultaneously development of research capacity is ensuring that local researchers can continue to monitor both people and the environment for the presence of antibiotic resistant pathogens into the future.

Author: Kim Summers, University of Warwick, United Kingdom

Co-Authors: Rangama, Séverine; Wellington, Elizabeth; Quince, Chris; Hashmi, Imran; Masood Ul Hassan, Sumara; Raguideau, Sebastien



Session B

Abstract 106

Poster Board number: 57

Challenges of outdoor air sampling for AMR surveillance

Background: Antimicrobial resistance (AMR) is abundantly present in wastewater and agricultural environments. Several activities like intensive farming, manure spreading on farmland, and wastewater treatment can generate bioaerosols containing antimicrobial-resistant microorganisms, carrying antibiotic resistance genes (ARGs). Bioaerosols can travel several kilometers. As such, a better understanding of AMR emissions from bioaerosols-generating activities would allow to identify and develop mitigation strategies. This study aimed to develop an outdoor AMR air sampling strategy suitable to monitor various bioaerosol-generating types of activities.

Procedure: Two high flowrate air sampling devices (SASS3100 at 300 L/min and SASS4100 at 4000 L/min) were used to collect upwind, on-site, and downwind of wastewater treatment plants, agricultural buildings, and during manure spreading activity in an experimental wind tunnel and on farmland. Two different control sites were also sampled. Samples were analyzed using qPCR for quantitation of total bacterial, fecal indicators, and 38 ARGs.

Findings: Several factors are influencing outdoor air collection efficiency 1) Weather conditions should be monitored during sampling. Rain is reducing particle concentration, and wind is causing bioaerosols dilution. 2) The geographical distribution of farms in Quebec province is making upwind controls difficult to manage. Upwind of a farm is downwind of another. Sampling on control sites should be privileged. 3) SASS4100 is underestimating concentration in the air by 7x factor compared to SASS3100. However, SASS4100 has a better sensitivity for ARGs detection since it collects 13x larger air volume. 4) Samples collected during spreading on farmland were on average 5x less concentrated compared to those collected during spreading in a wind tunnel.

Implications: We wish to present the challenges and limits of outdoor air sampling and to provide a comprehensive strategy for ARGs surveillance and evaluation of mitigation measures.

Author: Nathalie Turgeon, IUCPQ-UL, Canada

Co-Author: Baghdadi, Mahsa; Leclerc, Samantha; Bélanger Cayouette, Amélia; Brassard, Patrick; Godbout, Stéphane; Duchaine, Caroline

Back to the Program

Session B

Abstract 405

Poster Board number: 58

Monitoring antibiotic resistance genes in Austrian water systems and exploring links with (environmental) parameters

Background/Aim: Understanding antimicrobial resistance (AMR) in the aquatic environment is crucial for effective management strategies. This study aimed to monitor antibiotic resistance genes (ARGs) in Austrian water systems and investigate potential links with (environmental) factors.

Procedure/Method: Over two years (2019-2021), a comprehensive spatial and temporal sampling campaign was conducted across Austria, covering 26 surface waters (water, biofilm) and 20 wastewater treatment plants (WWTPs). Selected ARGs were monitored by qPCR and high-throughput qPCR (HT-qPCR). Additionally, various (environmental) factors including physical (e.g. temperature, pH, conductivity) and chemical parameters (e.g. nutrient levels, various trace pollutant concentrations incl. antibiotics) as well as microbial biodiversity (determined by amplicon sequencing) and others (e.g. anthropogenic impact, land use patterns, WWTP size) were considered to explore potential correlations with ARG prevalence and abundance.

Findings/Results: Preliminary results indicated a widespread distribution of ARGs, antibiotics and some other trace pollutants in Austrian water systems. Distinct ARG patterns could be detected in the different samples. ARG prevalence and abundance were found to be mostly higher in winter presumably due to increased infections and antibiotic prescriptions. Anthropogenic impact on ARG occurrence could be detected by qPCR in the surface water samples but not by HT-qPCR in the biofilm. So far, no clear trend of ARG occurrence with different biodiversity levels could be detected but additional qPCR analysis of the biofilm samples might be able to clarify potential correlations. Final results, including an in-depth correlation analysis to explore links with various (environmental) parameters will be presented at the conference.

Implications/Applications: The final results of this study will provide valuable insights into ARG prevalence and distribution in Austrian water systems. By exploring potential links with environmental factors, it will enhance our understanding of the complex dynamics driving AMR dissemination This knowledge will help to guide targeted interventions to mitigate the environmental spread of AMR.

Author: Julia Vierheilig, TU Wien, Austria

Co-Authors: Dielacher, Irina; Galazka, Sonia; Slipko, Katarzyna A.; Wögerbauer, Markus; Radu, Elena; Saracevic, Ernis; Klümper, Uli; Berendonk, Thomas U.; Krampe, Jörg; Kreuzinger, Norbert

Session B

Abstract 357

Poster Board number: 59

Investigation of Antibiotic-Resistant Escherichia coli Prevalence in Red Fox in a Rural Area of Northern Japan Using Culture-Method

Recently in rural Japan with rapid population decline, the accidental contact of residents with wild animals is reportedly increasing. Although wild animals have a potential to play an important role in circulation of antibiotic-resistant bacteria (ARB) especially in rural areas, less attention has been paid to them, compared to livestock animals, in one health approach. In this study, fecal samples of wild animals focusing on Red Fox were collected along forest trails four times from June to April 2023, in the Shonai region, Yamagata prefecture, a typical rural area in northern Japan. The collected samples (n=85) of animal feces were used for the isolation of E. coli using the selective agar and the identification of its origin by eDNA analysis. Among the 85 fecal samples, 33 samples (38.3%) were identified as the feces of Red Fox, while the origins of the other samples (n=52) were Japanese Marten, Masked Musang, and unknown. E. coli was detected in 24 of 85 fecal samples, and 66 strains, of which 27 strains were from Red Fox, were isolated from the 24 samples. The antibiotic susceptibility test demonstrated that 7 (10.6%) of 66 E. coli isolates had resistance to ABPC, TC, or ST, and all of the resistant isolates were obtained from 3 samples of Red Fox feces. Two of 7 resistant isolates were resistant to both ABPC and TC. This resistant rate was comparable to the other report (9.1%) on Red Fox in the other part of Japan, indicating the prevalence of antibiotic-resistant E. coli among this species of wild animal. All the 3 samples of feces positive for the resistant E. coli were collected in the areas close to the towns and villages, implying the fact that wild animals can acquire antibiotic resistance through access to human society.

Author: Masateru Nishiyama, Yamagata University, Japan

Co-Author: Watanabe, Toru; Takahashi, Ryota; Yoneda, Ichiro; Saito, Masayuki

Back to the Program

Session B

Abstract 193

Poster Board number: 60

Guide to strengthen monitoring and surveillance of AMR in farm animal environment: fostering a one health approach to integrated AMR surveillance

AMR is a major health threat to humans, animals, and environment; however, the majority of AMR surveillance efforts to reduce the spread of AMR have focused on human and animal components rather than the environmental sector. Recognizing the importance of the environmental sector under One Health, and regional needs, a regional guide on monitoring AMR in the farm animal environment (one of a series of Regional Guidelines for Monitoring and Surveillance of Antimicrobial Resistance (AMR), Use and Residues) is being developed through FAORAP.

Following a literature review, in March 2022, FAO RAP sent a survey questionnaire across the Asia Pacific Region. A draft guide was then developed which is undergoing country review in the region by technical experts. A revised version from FAO will undergo final consultations, including at EDAR7, and revision before publication.

In total, 41 surveys were received from 20 countries. Respondents indicated that they would be implementing projects on: spread of AMR from farms into the environment, introduction of AMR into farms and AMR circulation within farm - for both terrestrial production and aquaculture. The guidance most wanted by respondents was on how to design a sampling strategy and regional harmonization. Based on demand, the guidance covers surveillance design; sample collection; transport and processing; laboratory methods; data management and reporting.

There are too many knowledge gaps for a real surveillance system, so the guide will focus on supporting research. As farm animal environment AMR awareness and data increase, countries are struggling to implement sampling and analyse and contextualise their data. This guide will be an important step to integrate farm animal environment data into multi-sectoral data systems, and, along with the Quadripartite's risk prioritisation tools and national road maps for AMR and the environment, are helping countries to include environment in their AMR National Action Plans.

Author: Muhammad Usman Zaheer, Food and Agriculture Organization of the United Nations (FAO), Regional Office for Asia and the Pacific (RAP), Thailand

Co-Authors: Wagenaar, J.A. (Jaap); Gordoncillo, Mary Joy; Srisamran, Jutanat; Luiken, R.E.C. (Roosmarijn); Maluping, Ramon; Caro, Domingo; Sutherland, David

Session B

Abstract 195

Poster Board number: 61

Fate and Control of Clinically Relevant High-risk ARGs in Livestock-Related Environment

The livestock farming is one of the most important reservoirs of clinically relevant high-risk antibiotic resistance genes (ARGs), such as CTX-M-type extended spectrum β-lactamase genes (blaCTX-M), the ABC transporter gene conferring resistance to florfenicol and linezolid (optrA), and mobile tigecycline resistance gene (tet(X) variants, especially tet(X3/4)). The behavior and fate of these clinically important ARGs in animal manure treatment processes, particularly for aerobic composting and anaerobic digestion treatments, remains unclear. Herein, we found that above prevalent high-risk ARGs persisted in animal manure, anaerobic digestion and aerobic composting systems. Some high-risk ARGs, particularly blaCTX-M-14, tet(X4), and optrA, could even be detected in the unloading soil subsequent composting fertilizer application, which revealed the diffusion from animal manure to receiving soil. Furthermore, multiple tools, including the high-throughput sequencing, bioinformatic analysis, community-wide Bayesian Source Tracker method, and culture-based methods, revealed that temperature was the key factor affecting the persistence of high-risk ARGs and their bacteria hosts.The mesophilic (37 ◦C) or thermophilic (55 ◦C) anaerobic digestion, and thermophilic (> 55 ◦C) composting could reduce the abundance of most ARGs and inactivate the fecal bacteria significantly, while the blaCTX-M-14, tet(X4), and optrA remain even after the treaments. We constructed the hyper-thermophilic–mesophilic (70 ◦C-37 ◦C) two-stage anaerobic digestion system, and found the hyper-thermophilic anaerobic pretreatment and the two-stage process could block both vertical and horizontal transfers of blaCTX-M genes, optrA and mobile tet(X)-variants. Collectively, this study highlights the importance of controlling the environmental dissemination of clinically relevant high-risk ARGs by establishing a sound process and operational strategy.

Acknowledgement: This work was supported by National Natural Science Foundation of China (Grants 32141002).

Author: Ziming Han, Research Center for Eco-Environmental Sciences, Chinese Academy ofSciences, China

Co-Author: Zhang, Yu; Dai, Shiting; Liu, Shihai; Wang, Chen; Feng, Haodi; Yang, Min

Back to the Program

Session B

Abstract 185

Poster Board number: 62

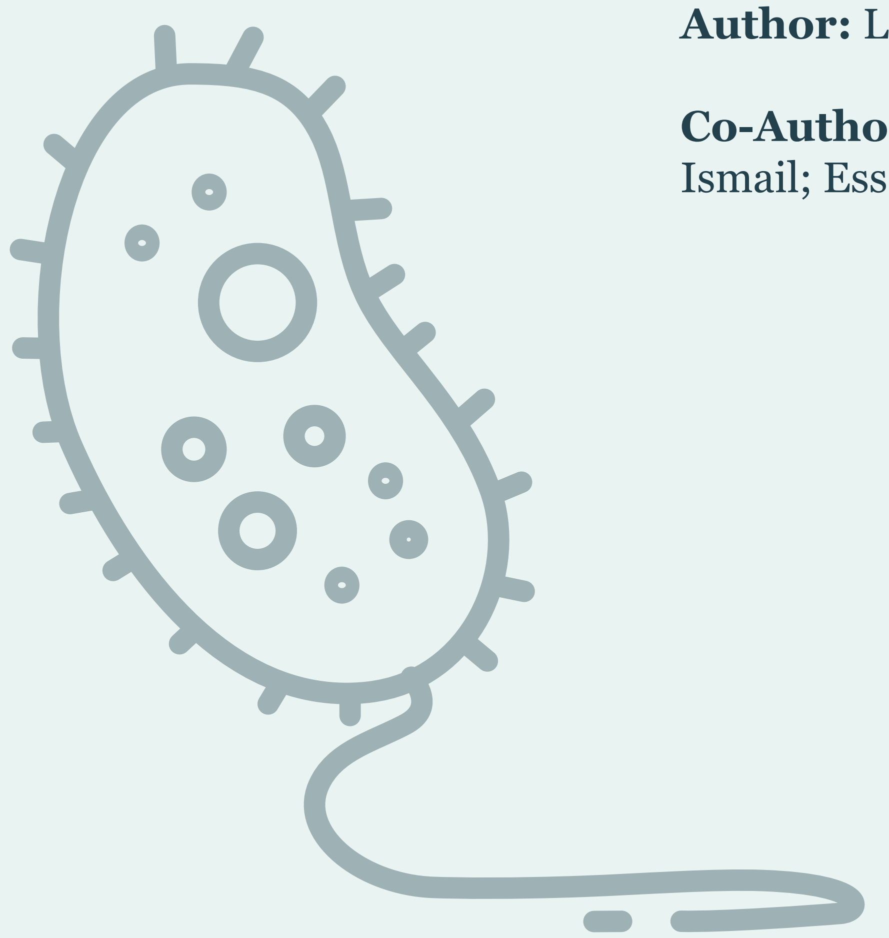
Flash Poster Presentation: S20 - Thursday, May 30 - 13:45-15:30

Pharmaceuticals at concentrations exceeding PNEC values in the aquatic environment fail to induce antimicrobial resistance in Escherichia coli

Most anthropogenically affected environments contain mixtures of pollutants from different sources. However, how these stressors contribute to the development of antimicrobial resistance in environmental microorganisms is poorly understood. This study quantified selected antibiotics, heavy metals, and biocides in an urban wastewater treatment plant (WWTP) and its receiving water body in Kwazulu-Natal, South Africa, vis-a-vis the predicted no-effect concentrations (PNEC) for the selection of antimicrobial resistance (AMR). Then, a 30-day exposure experiment to the environmental and sub-inhibitory concentrations of selected stressors (oxytetracycline, amoxicillin, zinc, copper, benzalkonium chloride 10 and diallyldimethylammonium chloride 12) was conducted using fully susceptible E. coli ATCC 25922 to ascertain any development of phenotypic or genotypic resistance. After 30 days, the exposed isolates were further subjected to extremely high doses of ampicillin to determine the development of persistence and tolerance to different concentrations of selected antibiotics, biocides, and heavy metals. Furthermore, wild-type isolates were collected from the same aquatic environment as the stressors and analysed for phenotypic resistance using the disk diffusion method and genotypically through whole genome sequencing. Most of the pollutants recorded values exceeding the PNEC values. However, exposure to the various stressors did not trigger phenotypic resistance in the experimental bacteria. Furthermore, WGS of the exposed isolates only revealed the macrolide resistance mdf(A) and disinfectant resistance genes sitABCD. Single nucleotide variants (SNV) mutations were detected for 19 genes encoding for oxidative stress, DNA repair, membrane proteins efflux systems, growth and persister formations except for the robA, which confers multidrug resistance in E. coli. This indicates that exposure to sub-inhibitory concentrations of antibiotics, heavy metals and biocide residues recorded in the current study could not induce phenotypic or genotypic resistance but encoded for genes responsible for the development of persistence and tolerance in bacteria, a potential precursor to the development of resistance in environmental bacteria.

Author: Luther King Abia Akebe, University of KwaZulu-Natal, South Africa

Co-Authors: Chukwu, Kelechi Benedict; Abafe, Ovokeroye A.; Amoako, Daniel G.; Arshad, Ismail; Essack, Sabiha Y.



Session B

Abstract 109

Poster Board number: 63

Flash Poster Presentation: S8 - Tuesday, May 28 - 11:00-12:30

Soil colloids affect the selective effect of different antibiotics on bacterial growth

Colloids (d < 1000 nm) form the small particulate phase of soils and consist of secondary minerals or of aggregates of inorganic and organic soil compounds. Due to their large specific surface, colloids can adsorb pharmaceutical residues entering soils irrigated with reclaimed water and, therefore, carry those residues to the groundwater and distribute them further in the environment (Xing et al., 2016). Also, colloids can adsorb larger proportions of nutrients and can change toxicity of inorganic and organic pollutants, which may influence the selective effect of pharmaceuticals on microorganisms (Missong et al., 2018). The bacterial growth response to different colloids as well as the potential buffering effect of colloids on the susceptibility of bacterial cultures during exposure to five selected antibiotics were tested. All experiments were performed in MH-broth as checkerboards. A standardized inoculum of five different species was added to each well. Optical density was measured for 18 hours during an incubation at 37 °C and horizontal shaking. For different combinations of antibiotics and colloids growth above the MIC of the tested strains was observed. Also, it could be shown that the colloids affect the growth of the tested strains differently. While gram-negative strains showed only minor growth inhibition, gram-positive strains were strongly inhibited. The results demonstrate the important influence of colloids on the behavior and efficiency of antibiotics. Especially in soils irrigated with reclaimed water, colloids may affect the availability of antibiotic residues and bacterial growth behavior. The results also raise questions about the behavior of pharmaceutical residues in soils with a long-term history of wastewater irrigation and how this may select for antibiotic resistances.

Missong et al., Phosphorus in water dispersible-colloids of forest soil profiles, Plant Soil 427 (2018)
Xing et al., Colloid-Mediated Transport of Pharmaceutical and Personal Care Products through Porous Media, Scientific reports 6, (2016)

Author: Katharina Axtmann, University Clinic Bonn, Germany

Co-Author: Brinkmann, Silas; Siskowski, Annette; Bierbaum, Gabriele; Heyde, Benjamin; Siemens, Jan; Braun, Melanie

Back to the Program

Session B

Abstract 170

Poster Board number: 64

Flash Poster Presentation: S8 - Tuesday, May 28 - 11:00-12:30

Distribution of antibiotic resistance between phages and bacteria in a river ecosystem receiving treated wastewater

Background and Aim: Transduction is well described in vitro but its contribution in disseminating antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) in the environment remains difficult to assess.

Procedure/Method: The distribution of 10 ARGs and 9 MGEs were studied in the bacterial and phage fractions collected from wastewater treatment plant (WWTP) effluents and receiving waters from the Meurthe River. Bacterial and phage DNAs were extracted separately, carefully checking that phage DNA extracts were not contaminated by free/non-encapsidated DNA. ARGs/MGEs and 16S rDNAs were then quantified by quantitative or digital PCR.

Findings/Results: Quantification of 16S rDNA in DNAs purified from phage fractions confirmed the presence of transducing phages containing chromosomal DNA in all the microbial communities analyzed. While in river waters, transducing phages were not very abundant and only carry a maximum of 0.08% of the total 16S rDNA content present in the whole microbial community, this value can reach 16.3% in WWTP effluents, thus demonstrating much higher content in transducing phages. This trend was also observed with the different ARGs/MGEs monitored where, for instance, a maximum of 0.01% of class 1 integrons were carried by phages in river communities, while up to 2% of them were carried by phages in the effluents. The propensity of ARGs/MGEs to be encapsidated was variable from one marker or one kind of water to another. Thus, in some effluents, transducing phages vehiculated more than the half of the sul1 genes and more than a quarter of the vanA genes! Although ARGs/MGEs were more abundant in effluents than the river, their abundances did not increase significantly in the river water downstream the WWTP.

Implications/Applications: These results notably arise the question of the impact the numerous transducing phages released in rivers through effluents with respect to their roles in disseminating ARGs/MGEs in the environment.

Author: Xavier Bellanger, LCPME UMR7564 CNRS-University of Lorraine, France

Co-Authors: Sagrillo, Charlène; Merlin, Christophe

Session B

Abstract 210

Poster Board number: 65

River Biofilms and AMR – Insights from Field and Laboratory Studies in Switzerland

Background and Aim: Rivers receive AMR contamination from wastewater, untreated sewage, and other sources. AMR in water mainly reflects current and potentially transient contamination. Biofilms on rocks or sediment of rivers contain sedentary microbiomes that are in continuous contact with their aquatic environment. They are thus potentially persistent reservoirs of AMR and potential sites of horizontal gene transfer. In several studies, we analyzed the resistome of river biofilms and studied biofilm invasion by resistant bacteria in laboratory experiments.

Procedure/Method: Natural biofilms on rock and sediment were studied in various locations in Switzerland and Europe, up- and downstream of WWTPs. The resistome of the biofilm communities was studied using qPCR and metagenomic approaches. Metagenomics on plating-enriched resistant phylotypes was coupled to environmental metagenomic data to trace resistant phylotypes. For experimental work, biofilms were grown in-river on glass slides, and exposed to model fecal bacteria invaders (genetically tagged E. coli) under controlled recirculation flow and environmental conditions in mini-flumes.

Findings/Results: Biofilm communities were found to harbor diverse resistomes, with considerable site-specific differences. Using qPCR and metagenomic approaches alone, a consistent effect of WWTP effluent on biofilm or sediment resistomes was not observed. However, phenotypic metagenomics revealed that sediments accumulated carbapenem-resistant phylotypes and biofilms accumulated ESBL phylotypes downstream of WWTPs. Flume Experiments showed that river grown biofilm can be invaded by the model invader. High temperature and stagnation did not impact persistence of invading E. coli, but disturbed biofilms appeared to support persistence of resistance plasmids or potentially horizontal gene transfer.

Implications/Applications: The lack of strong AMR accumulation in biofilms and abatement of AMR when wastewater exposure is removed suggests that biofilms are not likely to be general hotspots of AMR at levels of contamination encountered near Swiss WWTPs, but may nevertheless provide refuges for rare, but clinically relevant resistant bacteria.

Author: Helmut Bürgmann, Eawag: Swiss Federal Institute of Aquatic Science and Technology Switzerland

Co-Author: Gionchetta, Giulia; Lee, Jangwoo; Feng, Ju; Beck, Karin; ANTIVERSA consortium, The

Back to the Program

Session B

Abstract 227

Poster Board number: 66

A Framework for Modeling Microbial Ecological Alterations of the Resistome in the Sewershed

Background and Aim: The sewershed is a critical understudied microbial ecosystem in which multiple niches and processes can contribute to proliferation and attenuation of antimicrobial resistance (AMR). The purpose of this study was to develop a framework for understanding such processes in order to predict changes in the resistome as it travels from the source (e.g., human excreta and other household and industrial inputs) to the wastewater treatment plant (WWTP). Key factors to consider include: influx of antibiotic resistant bacteria, pathogens, resistance genes (ARGs), and mobile genetic elements (MGEs) from various sources; variations in diurnal, seasonal, and weather-related flow and their effects on biofilms and sediment; and the role of selective agents, such as antibiotics, antimicrobials, and heavy metals, in propagating AMR.

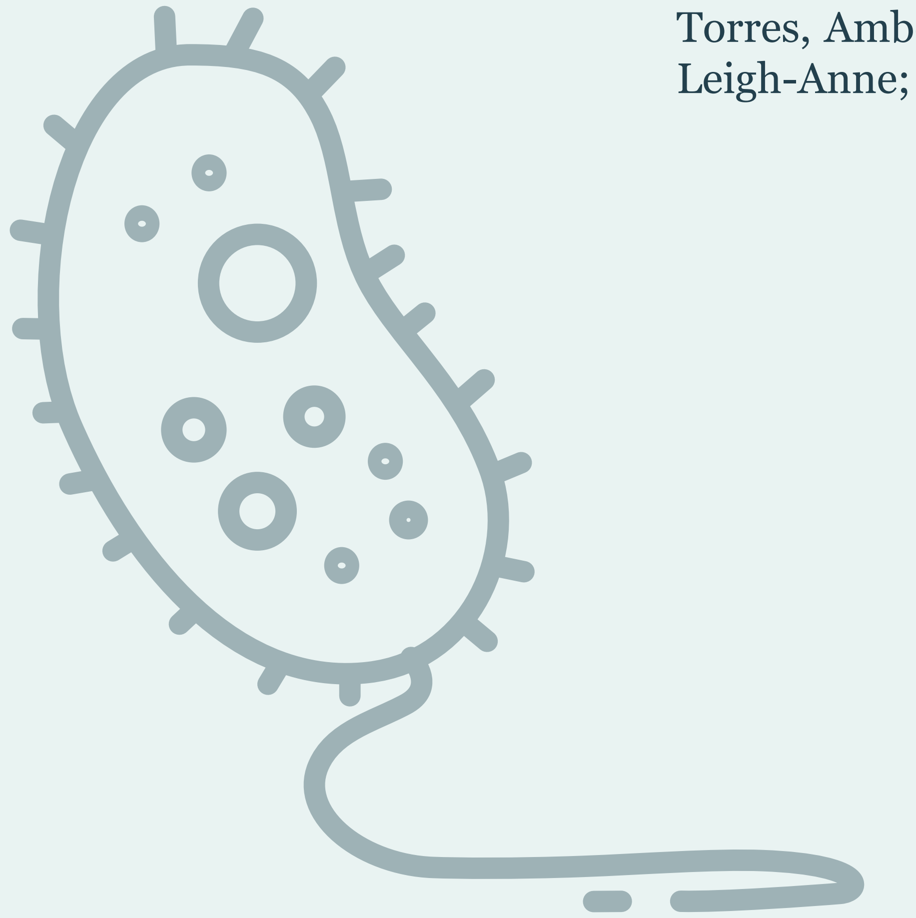
Procedure/Method: We selected a small sewershed compromised by aging infrastructure as an ideal model that includes multiple hypothetical sources and processes shaping the resistome to test the conceptual framework. An extensive 1-year sewershed sampling campaign was carried out, with the resistomes profiled via metagenomic sequencing, ddPCR, and culturing/whole-genome sequencing of cefotaxime-resistant Escherichia coli.

Findings/Results: Metagenomic profiles revealed key sources of ARGs and MGEs in the sewershed. It was found that inflow and infiltration not only diluted the sewage resistome signature, but also introduced external sources of ARGs and MGEs. A framework was developed to identify hypothetical sources and processes contributing to the observed shifts in the resistome throughout the sewershed. Neutral community models, partial least squares path models and metagenomic source-tracking approaches will be applied to test the framework.

Implications/Applications: This study provides new insight into key processes that shape the resistome of sewage as it travels through the network. The framework/models developed herein can be extended towards fine-tuning efforts to advance wastewater-based surveillance of AMR, taking into account how the sewershed can alter human fecal resistomes."

Author: Thomas Byrne, Virginia Tech, United States

Co-Authors: Darling, Amanda; Maldonado Rivera, Gabriel; Davis, Ben; Price, Sarah; Amaral-Torres, Amber; Markham, Clayton; Pruden, Amy; Deck, Madeline; Cohen, Alasdair; Krometis, Leigh-Anne; Vikesland, Peter



Session B

Abstract 356

Poster Board number: 67

Flash Poster Presentation: S20 - Thursday, May 30 - 13:45-15:30

The Minimum Ciprofloxacin Concentration Affecting the Microbial Community and Antimicrobial Resistance Gene Compositions of Anaerobic Processes

Background and Aim: Anaerobic digestion (AD) is employed for the reduction of wastewater treatment sludge and reuse of biosolids as fertilizers. The dissemination of antimicrobial resistance genes (ARGs) from wastewater to agricultural environments and their subsequent entry into the food chain is a rising concern. Ciprofloxacin frequently accumulates in AD processes near the minimal inhibitory concentrations for Escherichia coli. The goal of this study was to determine the lowest observed effect concentration in AD reactors.

Procedure/Method: Triplicate test and control reactors were operated for 208 days under mesophilic anaerobic conditions and fed either waste primary or secondary activated sludge. Subsequently, they were spiked with 0.0001, 0.1, 1 and 10 µg/mL of ciprofloxacin every 30 days. Microbial community composition was analyzed by 16S rRNA gene amplicon sequencing. ARGs and mobile genetic elements were quantified via qPCR. Fluoroquinolones in the sludge samples were quantified by ultrasonic extraction/HPLC-MS/MS.

Findings/Results: Microbial community compositions significantly diverged at ciprofloxacin concentrations above 1 µg/mL. The communities fed with primary sludge were more sensitive than the communities fed with secondary sludge based on Shannon index. Ciprofloxacin addition had varying impacts on different genes in both reactors. Overall, the gene copy numbers of the 95 ARGs analysed by qPCR arrays were significantly higher in the reactors fed with secondary sludge than those fed with primary sludge. The higher susceptibility of communities in primary sludge corresponds to the lower concentrations of naturally occurring fluoroquinolones.

Implications/Applications: The lowest observed effect concentration of ciprofloxacin that resulted in significant variations in community compositions were 10^4 to 10^5 times higher than predicted no effect concentrations for resistance (PNEC-R) reported in the literature. Several differences could be identified with literature experiments including length of the experiment and culture conditions. These findings underscore the need for broader investigation of different environments to fully understand the PNEC-R.

Author: Sukriye Celikkol, McGill University, Canada

Co-Author: Lafaille, Medinah; Kramer, Susanne; Caldwell, Catherine; Dinh, Quoc Tuc; Hassan, Zahra; Sauvé, Sébastien; Frigon, Dominic; Vo Duy, Sung

Back to the Program

Session B

Abstract 93

Poster Board number: 68

Community succession and antibiotic resistance in river biofilms on natural and artificial substrates

Background/Aim: Biofilms in aquatic ecosystems serve as environmental reservoirs of antibiotic resistance, but their importance remains poorly understood. This is due, in part, to lack of understanding regarding biofilm succession and ARG dynamics in situ. We addressed this knowledge gap by studying bacterial communities and ARG abundance in natural biofilms in James River (Virginia, USA). We considered succession during the initial colonization period (time scale of weeks to months) and longer-term community dynamics (bimonthly sampling over two years).

Methods: Natural biofilm samples were collected from rocks bimonthly. Whole community DNA was subjected to 16S amplicon sequencing and qPCR of multiple ARG. Water quality data, including temperature, pH, and dissolved oxygen, was collected daily over the study period. In addition, experiments were performed to examine shorter-term colonization dynamics by incubating glass slides and ceramic tiles in situ. Density (mass/unit area) and community composition of biofilms grown on artificial substrates were compared to natural biofilms.

Findings/Results: Seasonal differences in community composition were observed for the two-year natural biofilm samples. AR genes were consistently found in these samples, likely originating from urban wastewater overflow upstream. Colonization experiments showed markedly different results for slides versus tiles, with slides having faster biofilm growth and more similarity to the natural biofilm communities. After only one month, the community grown on slides was not significantly different from the natural biofilm, whereas the tile community was distinct for more than six months.

Implications: Our work indicates that biofilm succession should be considered in AR studies instead of utilizing snapshots and assuming a stable bacterial community. We also learned that colonization and early community succession are significantly impacted by the type of artificial substrate used; this has important implications for field experiments and laboratory microcosm studies seeking to understand how biofilms respond to environmental stressors.

Author: Mary Coughter, Virginia Commonwealth University, United States

Co-Author: Franklin, Rima

Session B

Abstract 207

Poster Board number: 69

Flash Poster Presentation: S17 - Thursday, May 30 - 11:00-12:30

Heavy metals increase antibiotic resistance gene maintenance in the environment

Background and Aim: Heavy metals can constitute a selective pressure on environmental bacteria and select for antibiotic resistance, as antibiotic resistance genes (ARGs) can co-occur with metal resistance genes, and some genes may confer resistance to both antibiotics and metals. Since metals persist longer than antibiotics in the environment, the selective pressure they impose on environmental bacteria could lead to an increased maintenance of ARGs in the environment. The aim of our study is to evaluate the role of heavy metals in environmental ARG maintenance in the absence of antibiotics.

Procedure/Method: River water microcosms were exposed to a combination of heavy metals (copper and zinc) at a sub-inhibitory concentration (50ng/ml) and to a combination of fluoroquinolone antibiotics (ciprofloxacin and ofloxacin) at the same sub-inhibitory concentration for 30 days. After 0, 2, 7, and 30 days of exposure to metal or antibiotic pollution, DNA was extracted to evaluate changes in bacterial community dynamics and ARG maintenance using 16S rRNA gene and metagenomic sequencing, respectively.

Findings/Results: Bacterial communities evolved differently over time under metal and antibiotic selective pressures. Efflux pumps conferring resistance to fluoroquinolones were detected by DNA similarity searches with specialized databases at a higher abundance in the metagenomic reads of samples exposed to metals than in those of samples exposed to antibiotics and of non-polluted controls. These results suggest that copper and zinc could maintain fluoroquinolone resistance in the environment in the absence of antibiotics. The ARGs that showed an increased relative abundance were different depending on whether antibiotics or metals were added.

Implications/Applications: Our results suggest that the long-term persistence of heavy metals in the environment could lead to the maintenance of ARGs in the environment, even in the absence of antibiotic pressure. Environmental metal pollution might therefore pose a risk to human health through exposure to ARGs.

Author: Emilie Dehon, Université Claude Bernard Lyon 1 and CHU de Québec-Université Laval Research Center, France

Co-Author: Mathieu, Alban; Droit, Arnaud; Vogel, Timothy M.; Sanchez-Cid, Concepcion

Back to the Program

Session B

Abstract 155

Poster Board number: 70

Characterization of Wastewater Treatment-Adapted Klebsiella pneumoniae: Implications for Environmental AMR and Pathogenicity

Background/Aim: Water treatment and sanitation are vital for infectious disease control. However, pathogenic microbes can evolve resistance to water treatment processes such as chlorine, UV, and heat, and microbes that survive wastewater treatment often exhibit antimicrobial resistance (AMR). Previous work discovered pathogenic E. coli in Alberta wastewater capable of resisting both wastewater treatment and antimicrobials. This project explored whether similar natural selection forces drive the evolution of pathogenesis and resistance (water treatment and antimicrobial) in Klebsiella pneumoniae in wastewater as well.

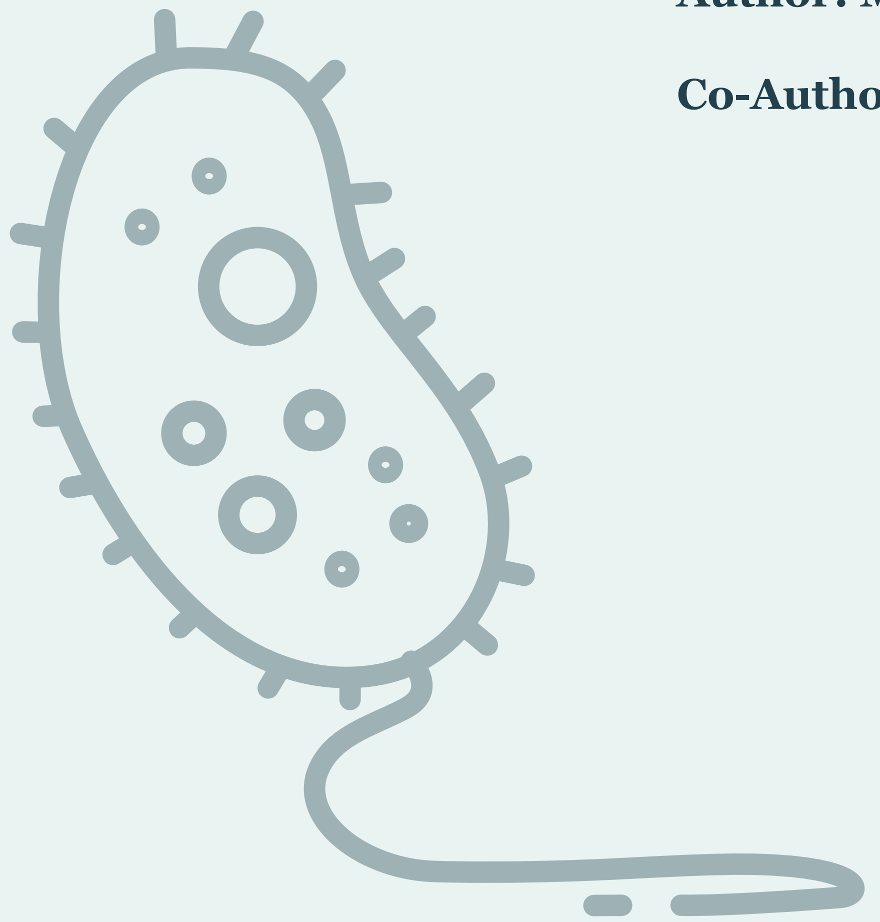
Methods: 26 K. pneumonaie samples were isolated from chlorine-treated sewage from Alberta wastewater treatment plants (WWTPs) and biochemically identified. The heat resistance of these isolates was tested by measuring the effects of a 5-minute 58°C heat treatment on strain culturability, and potential virulence was assessed using a string test for hypermucoviscosity (closely associated with a high virulence pathotype). Susceptibility to 17 clinical antimicrobials was evaluated using VITEK®2 ASTGN98 cards. Finally, the isolates underwent whole genome sequencing, with the goal of analyzing virulence and resistance genotypes.

Results: 18 K. pneumoniae isolates (76.9%) remained culturable after heat treatment. 6 isolates (23%), all culturable after heat treatment, tested positive for hypermucoviscosity. All 26 strains were broadly susceptible to the tested antimicrobials, with the notable exception of nitrofurantoin, against which 11 (42.31%) were resistant.

Implications: These 26 wastewater K. pneumoniae strains represented a chlorine-tolerant population among which resistance to heat treatment was also prevalent. Some isolates presented with a virulence-associated phenotype, but none exhibited high levels of AMR. Overall, these data suggest that pathogenic but antimicrobial susceptible K. pneumoniae strains may be evolving resistance to wastewater treatment. The release of these bacteria in WWTP effluents could threaten natural waters and downstream communities. Results of bioinformatic analyses are forthcoming and will be included in the presentation.

Author: Markus Gaenzle, University of Alberta, Canada

Co-Authors: Otto, Simon; Stothard, Paul; Neumann, Norman; Scott, Candis



Session B

Abstract 190

Poster Board number: 71

Analysis of antimicrobial resistance genes and plasmid transmissibility in Aeromonas spp. carrying blaIMP-1 isolated from hospital wastewater

Background and Aim: The spread of carbapenem resistance is a serious public health problem that requires measures based on One-Health approach. The horizontal transfer of plasmids with resistance genes can occur the spread of antimicrobial resistance. The aim of this study was to reveal the reservoir capability of Aeromonas spp. in hospital wastewater.

Method: Carbapenemase gene detection was performed on 794 strains of extended-spectrum cephalosporin-resistant Aeromonas spp. isolated from hospital wastewater in Japan. Multiplex PCR was performed to detect carbapenemase genes (blaIMP, blaVIM, blaNDM, blaKPC, and blaOXA-48). In addition, antimicrobial susceptibility testing, genomic epidemiology analysis, and plasmid transmissibility analysis were performed on the blaIMP-1 carrying Aeromonas strains. Escherichia coli and Aeromonas hydrophila were used as plasmid recipients.

Results: Out of 794 isolates, 54 strains were carrying blaIMP-1. MLST analysis classified the 54 strains into 19 novel sequence types. Antimicrobial susceptibility testing revealed that many strains were resistant to meropenem and imipenem. Plasmids carrying blaIMP-1 were transferred by conjugation to the recipient Aeromonas hydrophila in 2 of the 54 isolates. Plasmids analysis showed that integron cassettes were located on IncP-1 plasmids. Comparison of the two strains showed similar genetic structure around the blaIMP-1.

Implications: The blaIMP-1 carrying Aeromonas spp. were predominantly detected in hospital wastewater in this study, confirming the plasmid-mediated transmission of the blaIMP-1. This result supports the role of Aeromonas spp. as a reservoir of antimicrobial resistance genes in wastewater, which is emerging research. Further analysis focusing on antimicrobial resistance plasmids is necessary to investigate the mode of horizontal transmission of resistance genes in Aeromonas spp.

Author: Mako Koyama, Kitasato University, Japan

Co-Author: Niida, Nagi; Eda, Ryotaro; Suzuki, Masato; Izawa, Hiroki; Amarasiri, Mohan; Furukawa, Takashi; Sei, Kazunari; Kubo, Makoto; Maehana, Shotaro

Back to the Program

Session B

Abstract 138

Poster Board number: 72

Assessment of antibiotic resistance across the agro-ecosystem

Background and Aim: Agricultural practices regarding the use of antibiotics and other biocides have repercussions for human, animal, and plant health. The aim of this study was to evaluate the frequency of antibiotic resistance across the agro-ecosystem on an antibiotic-free swine farm.

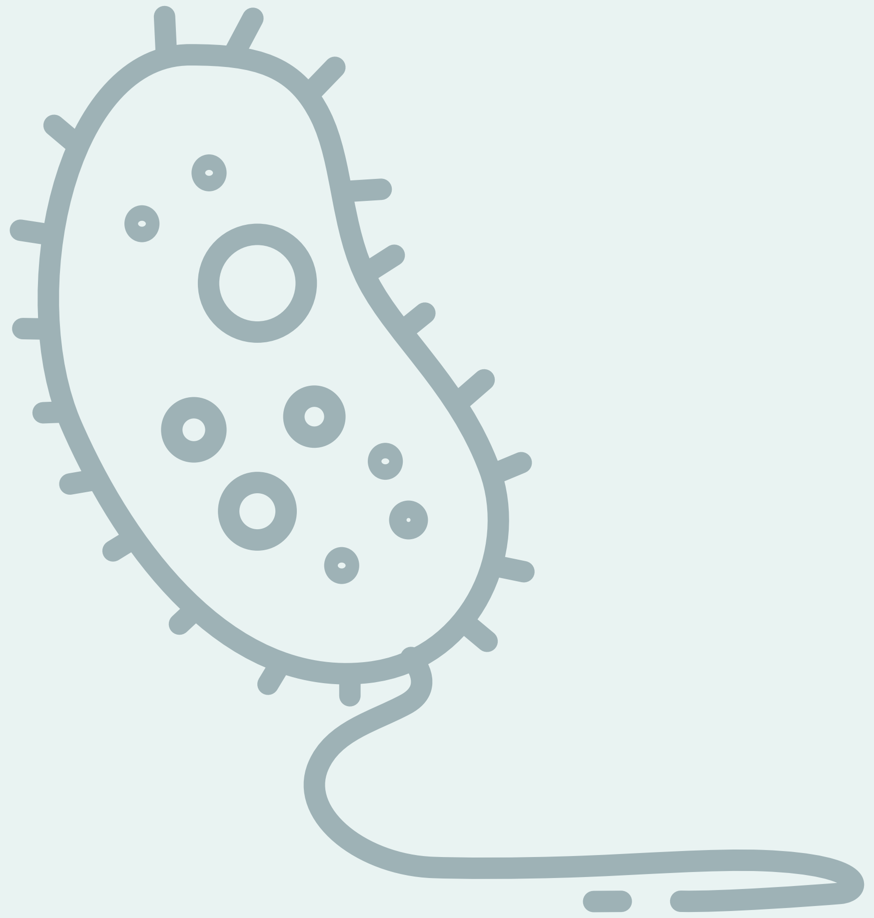
Method: Samples of feed, feces, manure, agricultural and forest soils, surface water, and sediments were collected in fall 2022, spring 2023, and fall 2023. All samples were subjected to counts of total Enterobacteriaceae, and the sub-populations resistant to cefotaxime (CTX) or ciprofloxacin (CIP), as well as total Enterococcus and Enterococcus resistant to vancomycin (VAN). The frequencies of antibiotic resistance genes (ARGs), of importance to human health, were determined using high-throughput qPCR in a subset of feces and soil samples.

Results: Frequencies of total Enterobacteriaceae, Enterococcus, and ARGs were higher in piglet feces than in the feces of older pigs. Resistance to CIP was observed in feces, agricultural soil, water, and sediments at relatively low levels, while a large proportion of Enterobacteriaceae in pig feces and in environmental samples were resistant to CTX. VAN-resistant Enterococcus were recovered from feces, but not found in the environmental matrices. Even though VAN-resistant Enterococcus were found in manure samples, none were detected in the soil 14 days after manure application. The frequency of ARGs was greater in soil than in feces, where vancomycin RGs showed an increased ratio in soil. In contrast, tetracycline RGs were found in elevated proportions in feces.

Implications: Antibiotic resistant bacteria were prevalent in pig feces, even without the administration of antibiotics, which suggests that other factors contribute to their presence. Increased CTX resistance was seen in forest soils as well as in water and sediments upstream of the farm, suggesting alternative resistance sources. This study sheds light on the complexity of components influencing antimicrobial resistance in the agro-landscape.

Author: Alexandra Langlois, Agriculture and Agri-Food Canada, Canada

Co-Authors: Vincent, Antony T.; Petri, Renée; Lauzon, Karoline; Poulin-Laprade, Dominic



Session B

Abstract 52

Poster Board number: 73

Flash Poster Presentation: S18 - Thursday, May 30 - 11:00-12:30

Longitudinal ARGs Profiles in Wastewater Treatment Plant Influent Revealed by Metagenomic Analysis

Imprudent antibiotic usage accelerates antimicrobial resistance caused by antibiotic-resistant bacteria and antibiotic-resistance genes, thereby endangering human and animal health. Sewage influents of wastewater treatment plants (WWTPs), containing thousands of people's gut microbiomes, are considered antibiotic-resistant hotspots. Although spatial studies have examined WWTP influents from diverse regions and countries to assess their resistomes, longitudinal studies are lacking. This work examined temporal ARG profiles of Shatin WWTP influent in 13 years using metagenomics analysis of 97 monthly samples. The results show that the total ARG abundance significantly changed every three to four years, Cluster 1 (2010.10–2013.12), Cluster 2 (2014.01–2017.12), and Cluster 3 (2018.01–2022.03), with fluctuation in the range of 0.50~2.08 copies/cell, and eleven ARG types increased in abundance and proportion over the years. Furthermore, PCoA analysis revealed a distinct resistome composition in Shatin sewage influent compared to various sample types, including human and animal specimens and other sewage samples. Through network examination, strong co-occurring connections were discovered among 71 specific Class one integrons and 198 subtypes of ARGs originating from 13 types, demonstrating their potential for horizontal gene transfer. Co-occurrence patterns were mostly found among related ARG types or between two types, like beta-lactam and multidrug. Furthermore, the results also showed that 775 species from seven phyla could potentially serve as hosts for 196 subtypes of ARGs of 15 types. This longitudinal study used an 'omics-based' approach to assess high-risk ARGs' medical threats. The findings revealed that clusters 1 and 2 exhibited a seasonal increased risk for AMR. However, after implementing policies aimed at controlling AMR in Hong Kong, cluster 3's risk returned to pre-policy levels, signifying the efficacy of such interventions in reducing AMR. The findings in this longitudinal influent analysis provided insights into the risk assessment of sewage resistome regarding AMR threats to human health.

Author: Shuxian LI, University of Hong Kong, China

Co-Author: ZHANG, Tong

Back to the Program

Session B

Abstract 6

Poster Board number: 74

Prophage induction by non-antibiotic compounds promotes transformation of released antibiotic resistance genes from cell lysis

Background and Aim: Prophage is prevalent among bacterial species, including clinical pathogens that carry antibiotic-resistance genes (ARGs). Prophage induction can be triggered by the SOS response after detecting DNA damage, thus causing cell lysis. Previous studies suggest non-antibiotic compounds could induce bacterial DNA damage. However, it is unknown whether they could trigger prophage induction, and subsequently promote the exchange of ARGs released from cell lysis.

Procedure/Method: In this study, we combined bacterial culturing, flow cytometer-based phage and oxidative stress detection, transmission electron microscope (TEM) imaging, and quantification of RNA transcription to investigate the effects of non-antibiotic compounds triclosan and silver nanoparticles on triggering prophage induction in clinical ESKAPE isolates carrying ARGs, as well as the subsequent uptake of released ARGs by naturally competent bacteria.

Findings/Results: Our results demonstrate that both triclosan and silver nanoparticles from environmentally relevant concentrations to concentrations found in commercial products, could significantly enhance prophage induction for up to 5.6 folds among various clinical isolates. TEM imaging and plaque assays confirmed the production of infectious phage particles resulting from prophage induction mediated by non-antibiotic compounds. Moreover, the rate of ARG transformation to Acinetobacter baylyi significantly increased by up to 2.5 folds after the release of extracellular ARGs from prophage induction-mediated cell lysis. The mechanism of non-antibiotic compounds mediated prophage induction is primarily associated with excessive oxidative stress, followed by the provoked SOS response.

Implications/Applications: Our findings demonstrated that non-antibiotics TCS and AgNPs can promote the cross-species transformation of ARGs by mediating prophage induction and lysing the host ESKAPE strain. This study advances our ecological understanding of the spread of antimicrobial resistance in diverse bacterial species. Considering the prevalence of prophages identified in clinically important antibiotic-resistant pathogens and a broader range of bacterial species, our findings raise concerns regarding the potential antibiotic-like roles induced by non-antibiotics.

Author: Jianhua Guo, University of Queensland-AWMC, Australia

Co-Authors: Lu, Ji

Session B

Abstract 325

Poster Board number: 75

Flash Poster Presentation: S19 - Thursday, May 30 - 13:45-15:30

The role of synanthropic birds as sources and potential transmission agents of AMR in Uganda

Background: Synanthropic migratory birds forage for food from landfills, abattoirs and surface waters, increasing the risk of carriage and transmission of antimicrobial resistance (AMR) in vast environmental settings. Birds amplify the AMR challenge by spreading pathogens like Enterobacteriaceae, treated using beta-lactams. Pathogens often come into contact with antibiotic resistance genes carried by birds and develop resistance to antibiotics like beta-lactams. Consequently, Extended-spectrum beta-lactamases (ESBL) and plasmid-associated genes cause resistance against beta-lactams. PAIRWISE consortium determined the role of synanthropic birds in carriage and transmission of ESBL-producing Escherichia coli and Klebsiella pneumoniae in different habitats.

Methods: Bird (marabou storks, pied crows, hamerkops, and grey-headed gulls) faecal droppings were picked from abattoirs and landfills (Gulu and Mbarara), and Lake Mburo landing site. CHROMagar-ESBL medium was used for phenotypic characterization of E. coli and K. pneumoniae. Positive isolates were identified using Vitek 2 System. Genomic DNA was extracted from ESBL-resistant bacteria using ZymoBIOMICS DNA prep kit, followed by PCR amplification of genes, blaTEM, blaSHV and blaCTX-M previously isolated from clinical sources in Uganda.

Results: Of the 70 isolates identified, 17 were K. pneumoniae; 21 E. coli; the rest categorized as “others” (e.g., Citrobacter freundii, Pantoea). The majority of resistant isolates (38/70) were ESBL-producing. The gene blaCTX-M was amplified from 37 ESBL-producing E. coli and K. pneumoniae isolates, blaTEM from 21, and blaSHV from 14 isolates.

Discussion: The study demonstrates the first evidence of the role of synanthropic birds as sources and transmission agents of AMR in Uganda. Foraging behaviour of the birds from abattoirs, landfills and landing sites amplify the risks of spread of AMR.

Conclusion: The risk of spread of AMR by synanthropic birds is enhanced by surface water bodies in proximity to landfills, abattoirs and fish landing site. The findings guide the implementation of policies on AMR control through the one-health approach.

Author: Charles Masembe, Odong, Robinson, Makerere University, Uganda

Co-Author: Mayega, Johnson Francis; Akoll, Peter; Omara, John; Katende, George; Ndinawe, Ruth Pamela

Back to the Program

Session B

Abstract 293

Poster Board number: 77

Microbiome and Resistome within a First Nation Community and its Surrounding Aquatic Ecosystem in Manitoba, Canada

Background and Aim: The increasing resistance of bacteria to antibiotics is one of the biggest threats to public health in the 21st century. Each year, antibiotic resistance leads to thousands of deaths around the world. The continuous release of antibiotic-resistant genes into the water by human and agricultural waste makes water the primary method of spreading antibiotic-resistant bacteria, residues of antibiotics, and mobile genetic elements (MGEs). My aims of the project are- 1. To develop microbiome fingerprints of wastewater facilities to examine seasonal changes and perturbations following different treatment processes, 2. To assess the contribution of Mobile Genetic Elements (MGEs) via functional metagenomics, and 3. To investigate the impacts of the disinfection process in bacteriophage ARGs and the extent of ARG transfer from phages during wastewater treatments occurring in First Nation communities of Manitoba, Canada.

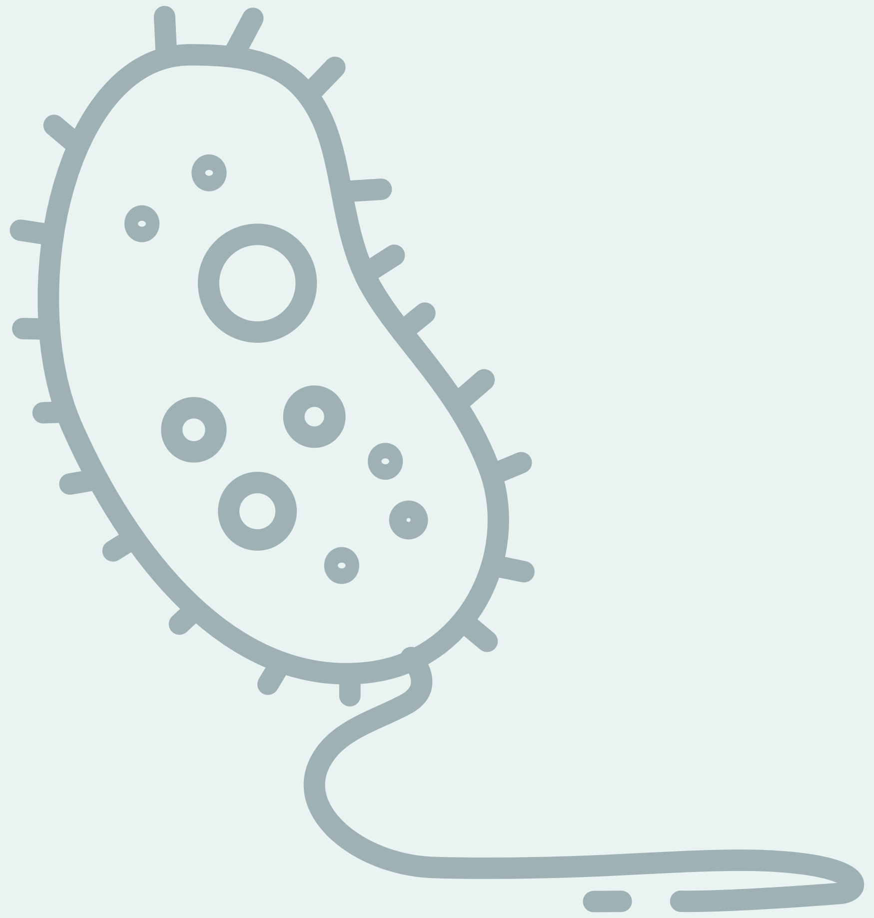
Method: I use Oxford Nanopore Technology (ONT) and function-based metagenomics combined with culture-independent approaches to study the bacterial and phage communities. HMW-DNA is extracted and used for sequence-based and functional metagenomics to reveal ARGs. The use of ONT provides longer sequences and thus more informative reads.

Results: I sequenced 70 (35 phages + 35 bacteria) samples (from 5 different points 7 times) and got around 700 GB of raw data after sequencing. I will start analyzing this using Kraken2 and other bioinformatic tools such as Metaflye, MG-RAST, and CARD.

Implications/Applications: The goal of our project is to evaluate UV treatments (pulses and time exposure) in the wastewater treatment process from a microbiological perspective. UV treatments could help improve to explain how the dynamics and interactions between bacteria and phages are affected by the most common disinfection process. Our research findings will ultimately guide future water monitoring and treatment processes to minimize the escape of resistant pathogens into one of our most valuable natural resources, water.

Author: Md Moniruzzaman, University of Manitoba, Canada

Co-Author: Uyaguari, Miguel



Session B

Abstract 330

Poster Board number: 78

Flash Poster Presentation: S8 - Tuesday, May 28 - 11:00-12:30

Detection of plasmid-borne copper resistance genes in clinical isolate of Legionella pneumophila using comparative genomics

Background and Aim: Legionella pneumophila is an opportunistic environmental bacterium and clinical pathogen responsible for several life-threatening outbreaks of Legionnaires' disease (LD), a severe form of pneumonia. L. pneumophila sequence type ST378 seems to be endemic in Montréal as it has been isolated from healthcare facilities as well as associated hot water distribution systems (HWDS) over the last 15 years. The goal of this study was to determine the diversity of L. pneumophila ST378 environmental and clinical isolates using whole genome sequencing (WGS) and comparative genome analysis.

Method: Short- and long-read sequencing was used to produce high quality closed genomes of ST378 isolates. KSNP4 was used for phylogenetic analysis using single nucleotide polymorphisms (SNPs). Pangenome analysis was performed with Roary.

Results: Genomic analysis of the clinical and environmental ST378 isolates revealed 315 core SNPs. An 89 kbp plasmid was detected in a clinical isolate presumably acquired through horizontal gene transfer. High concentration of copper was detected in the HWDS associated with this case. The plasmid harbors an extra copy of copA and mmcO genes. CopA encodes Copper-exporting P-type ATPase and mmcO encodes a copper monooxygenase involved in copper resistance, and increased copper resistance was confirmed experimentally. Further analysis of this ST378 isolate's genomes using Resistance Gene Identifier against the Comprehensive Antibiotic Resistance Database predicted genes encoding antibiotic resistance against macrolides, fluoroquinolones, and tetracyclines, some of them carried on the plasmid.

Implications: High level of copper in HWDS selected for increased copper resistance and promoted evolution of Lp in HWDS. Our study further supports previous observation that resistance to heavy metals can promote propagation of antimicrobial resistance genes and evolution of opportunistic pathogens in the built environment.

Author: Maria Najeeb, McGill University, Canada

Co-Author: Matthews, Sara; Prevost, Michele; Brodeur, Julie; Cadieux, Geneviève; Pilon, Pierre; Lalancette, Cindy; Faucher, Sebastien P.

Back to the Program

Session B

Abstract 136

Poster Board number: 79

Antibiotic-resistance genes in bioerosols from wastewater treatment plants: emission and control by air biofiltration

Background and Aim. Wastewater treatment plants (WWTPs) can be hotspots for antibiotic-resistant bacteria (ARBs) and genes (ARGs) and are confirmed sources of bioaerosols. Their dispersion into the environment can present a health risk for workers. In some indoor WWTPs, air is collected directly at the process and sent to a biofiltration system for odor control, prior to its release into the environment. The objectives of this study are to: 1) evaluate the capacity of an odor biofiltration system to reduce the microbial and ARG concentrations in WWTP treated air and 2) evaluate bacterial and ARG aerosolization from water to air according to different wastewater treatment processes.

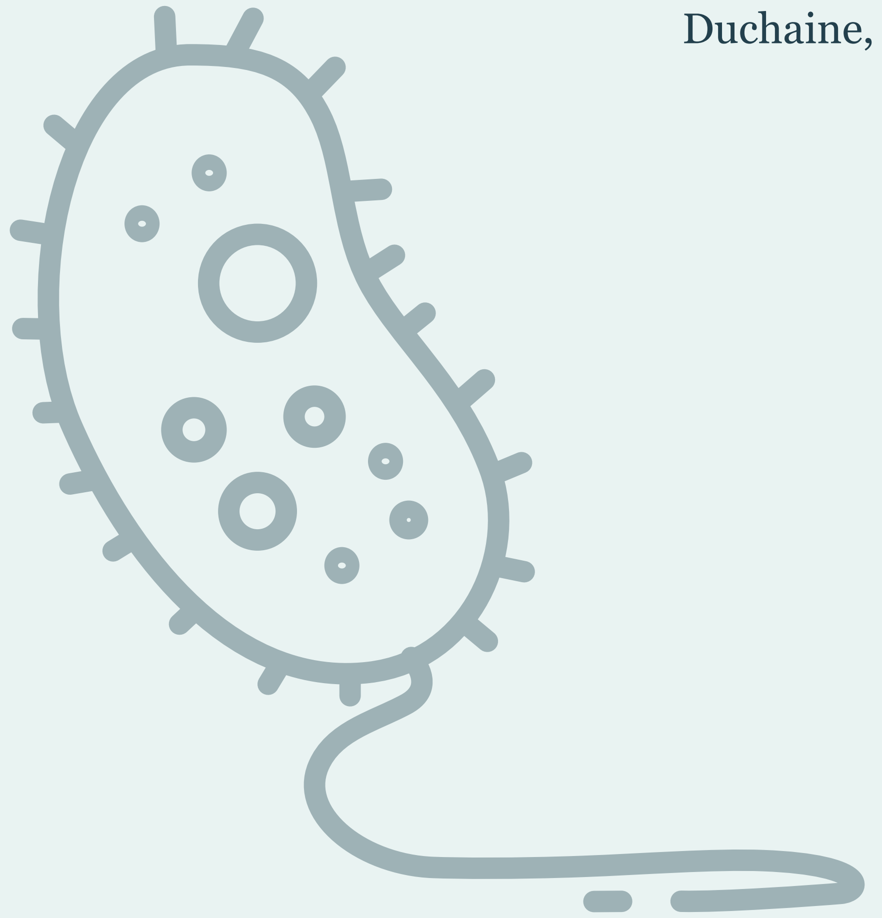
Method. First, 28 air samples were collected before and after biofiltration treatment in an interior WWTP in Quebec, Canada. Total bacteria and 35 ARGs encoding resistance to beta-lactams, aminoglycosides, glycopeptides, quinolones, sulfonamides, tetracyclines, polymyxin, and macrolides were quantified by quantitative Polymerase Chain Reaction (qPCR). Water and air samples were then collected from high bioaerosol-generating processes in 15 WWTPs. Total bacteria and 16 ARGs will be quantified in 25 selected air/water paired samples.

Results. Overall, higher total bacterial concentrations were detected in air samples prior to treatment. 28 ARGs were detected and quantified before the biofiltration system, whereas 24 ARGs were still detectable and quantifiable after treatment. Of those, 13 exhibited a significant reduction in their relative abundance, and none were significantly higher in the effluent. Tetracycline resistance genes were detected with the highest abundance in the air samples collected before and after the biofilter, whereas vancomycin resistance gene were not detected at all. Detection of ARGs in paired air/water samples is currently in progress.

Applications. Overall, these results show the benefits of biofiltration air treatment to reduce bacterial and antimicrobial resistance in air, a promising application to limit environmental dispersion and workers exposure.

Author: Arthur Ouradou, Polytechnique Montréal, Canada

Co-Authors: Bélanger-Cayouette, Amélia; Khaddouma, Salim; Lachance, Emie; Veillette, Marc; Duchaine, Caroline; Bédard, Émilie



Session B

Abstract 270

Poster Board number: 80

Flash Poster Presentation: S8 - Tuesday, May 28 - 11:00-12:30

New epicPCR based system allows tracking of horizontal gene transfer of multiple resistance genes in a microbial community simultaneously

Background and Aim: Horizontal gene transfer (HGT) plays a key role in the spread of antimicrobial resistance genes (ARGs) and we need to understand its different aspects to mitigate resistance problem. Until now, we couldn't follow multiple individual genes in complex natural communities and see how they spread or what factors affect it. Our objective was to develop a system to track the HGT of these genes.

Method: We developed an Emulsion, Paired-Isolation and Concatenation PCR (epicPCR) based method, which allows us to track the HGT of multiple genes or gene copies simultaneously in a microbial community. We first used it to follow the natural spread of multiple copies of an ARG with different origins in an artificial microbial community in liquid microcosms. The genes were introduced into the community in three genetic contexts both in a live host and as an extracted DNA. To test the system with this first experiment, we researched the effect of temperature on HGT as it has been shown to correlate with higher clinical resistance load.

Results: We were able to distinguish the six gene copies from each other and found to which species they had spread to at the end of the experiment. We saw that temperature can have an effect to which and to how many species the genes spread to. As could be presumed, there were differences in how the gene copies reached new hosts based on their origin.

Applications: This system opens possibilities to new kind of research towards more detailed understanding of HGT of ARGs in natural bacterial communities. It allows us to expand our knowledge in how these genes spread in complex communities as well as the importance of different ways of HGT in the big picture.

Author: Veera Partanen, University of Helsinki, Finland

Co-Author: Virta, Marko; Hiltunen, Teppo; Muurinen, Johanna; Karkman, Antti; Dekić Rozman, Svjetlana

Back to the Program

Session B

Abstract 249

Poster Board number: 81

Flash Poster Presentation: S10 - Tuesday, May 28 - 13:45-15:30

Comparative genomics of Escherichia coli ST131 of human, animal and environmental origin from the Czech Republic

Background and Aim: Escherichia coli and its multidrug-resistant sequence types (ST) are successful at spreading morbidity, mortality, and antibiotic resistance. One of the most widespread clones of medical importance is pandemic extraintestinal pathogen ST131 which is responsible for human nosocomial diseases and animal infections and disseminates through various environments. The aim of the study was to explore genetic structure of a large collection of ST131 in terms of antibiotic resistance genes and plasmids and perform phylogenetic analysis to evaluate the clonality of isolates collected from different sources.

Methods: We performed a whole-genome sequencing of 898 ST131 isolates originating from humans (n=713), the environment (n=139), wildlife (n=32), food animals (n=13) and companion animals (n=1) in the Czech Republic during 2009–2021. The content of antibiotic resistance genes, virulence factors and plasmid replicons was assessed. Core-genome-based phylogenetic analysis enabled categorization of strains into clades A, B, C0, C1 and C2.

Results: We determined 72.4% of isolates carried a variant of blaCTX-M responsible for resistance to cephalosporins regardless of the clade or source. Most isolates belonged to clade C (804/898), characterized by fimH30 variant, with C1 (262/898) carrying blaCTX-M-27 and C2 (528/898) linked with blaCTX-M-15. Within C1 clade, high clonality was observed among a subset originating from humans, wild birds, and wastewater; a trend also observed in clade C2 within groups obtained from humans and wastewater. Presence of various F plasmids was detected regardless of the clade association.

Implications: Our analysis demonstrates high clonality amongst ST131 isolates from various sources, especially in clades C1 and C2, highlighting their importance within a One Health context. Most of the isolates were associated with diverse epidemiologically relevant F plasmids. These findings show the importance of studies focused on previously unmonitored parts of the world and support the claim of the global threat these bacteria pose.

Author: Michaela Ruzickova, University of Veterinary Sciences Brno, Czechia

Co-Authors: Jamborova, Ivana; Nohejl, Tomas; Sukkar, Iva; Palkovicova, Jana; Papousek, Ivo; Cummins, Max; Djordjevic, Steven; Dolejska, Monika

Session B

Abstract 176

Poster Board number: 82

Antibiotic resistance is maintained in anthropogenically-polluted aquatic ecosystems in the absence of further selective pressure

Background and Aim: Anthropogenic pollution may select for antibiotic resistance (AR) in environmental settings. Genes associated with AR might provide a selective advantage and be maintained in the environment in the absence of antibiotics. The goal of this study was to determine the extent of AR maintenance in anthropogenically-polluted surface water in the absence of further selective pressure.

Procedure/Method: Microcosms (19L) of river water were polluted with 1L of treated wastewater. In parallel, 20L river water microcosms were used as controls. Duplicates were incubated at room temperature and at 4 °C for 70 days and sampled 3 times/week for the first 3 weeks and once every 2 weeks until day 70. A combination of culture-based (quantification of total and ESBL-producing E. coli and oligotrophic bacteria) and molecular approaches (ARG-qPCR and metagenomic resistome screening) was used to determine the maintenance of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in contaminated surface water.

Findings/Results: Total E. coli were maintained for longer periods of time in the cold. However, ESBL-producing E. coli disappeared during the first week at all temperatures. On the other hand, oligotrophic bacteria (total and ESBL-producing) were maintained during the experiments regardless of incubation temperature. Overall, ARGs were maintained in all the samples. The relative abundance of total ARGs and genes involved in tetracycline, macrolide and multidrug resistance was constant through the experiment, however, bla-OXA48 abundance increased over time. In addition, wastewater pollution had a limited overall impact on resistome composition and abundance in the receiving waters.

Implications/Applications: The environmental pool of ARB and ARGs is maintained for long periods of time in the absence of further selective pressure, whereas human pollution showed a limited impact on this environmental AR background. This research highlights the risk associated to AR maintenance in environmental settings.”

Author: Concepcion Sanchez-Cid, Université Claude Bernard Lyon 1, France

Co-Author: Stange, Claudia; Dehon, Emilie; Vogel, Timothy M.; Tiehm, Andreas

Back to the Program

Session B
Abstract 65

Poster Board number: 83

Do differences in health vulnerability indices between two sewersheds basin impact antimicrobial resistance in sewage of a major Brazil's capital?

Background and Aim: Given the connections between antimicrobial resistance (AR) and sanitation, there is an inverse correlation between the indicators of these themes1,2. In Belo Horizonte, major capital in Brazil, some of these indicators are used to calculate the Health Vulnerability Index (HVI)3, which is responsible for classifying areas of the sewersheds basins of Arrudas and Onça as low-medium and medium-high4. Thus, the present study evaluated, in Belo Horizonte, the expression and impact of the difference between HVI on the dynamics of AR through quantifying antibiotic resistant bacteria (ARB) abundance in sewage samples.

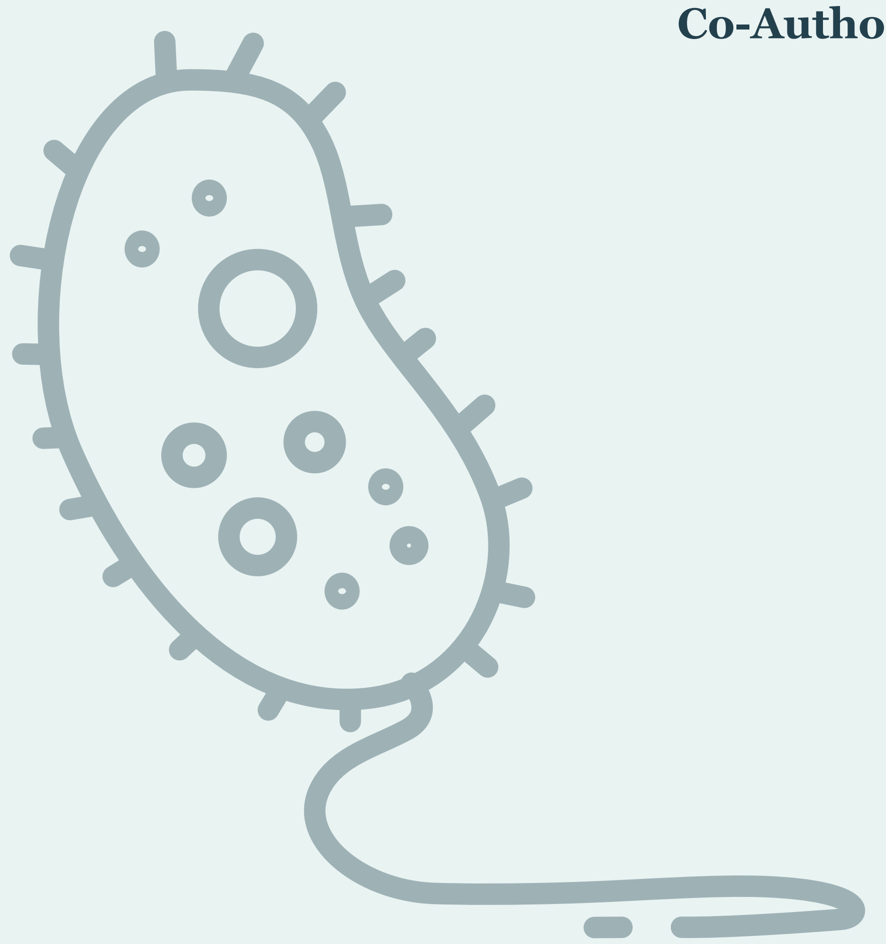
Procedure/Method: In view of the objective, the Mann-Whitney hypothesis test was carried out, according to data sets determined by Coelho5 and Machado6, over 14 months (2020; 2021; 2022), which contain the concentration of ARB resistant to the amoxicillin, azithromycin, cephalexin, triazole (sulfamethoxazole + trimethoprim) and meropenem in the raw sewage of the aforementioned basins.

Findings/Results: Although the HVI of the areas are different, no statistically significant difference was detected in the median concentration of ARBs in sewage, except for meropenem (7.8 x 104 UFC.ml-1 and 4.3 x 104 UFC.ml-1 to Arrudas and Onça, respectively). This result can be discussed based on the possible lack of impact of the variables that make up the HVI on one of the main factors impacting the RA: drug consumption7. The difference found for bacteria resistant to meropenem, an antibiotic for hospital use, corroborates the massive number of hospitals in the Arrudas basin, which probably made its concentration higher in relation to the Onça basin.

Implications/Applications: The results provide insights to stakeholders, as they could monitor the RA in raw sewage of just one basin, in addition to designing unique actions for both locations, while these do not have changes in the dynamics of dissemination of resistance to large most antibiotics.

Author: Juliana Calábria de Araújo, Universidade Federal de Minas Gerais, Brazil

Co-Author: Santos, Henrique



Session B
Abstract 267

Poster Board number: 84

Wastewater borne pollutants affected the soil resistome and mobilome but not the overall microbial community composition

Background and Aim: Irrigation with wastewater mitigates water scarcity and promotes agricultural productivity. However, pollutants in the wastewater can favor the environmental spread of antimicrobial resistance. We investigated how the shift from irrigation with untreated to treated wastewater affected the abundance and diversity of ARGs and MGEs and associated microbiome in soils irrigated over 80 years with untreated wastewater.

Method: A soil incubation experiment was conducted using three different soil types exposed to treated or untreated wastewater each spiked and not spiked with antibiotic and disinfectants. Samples were taken four days and four weeks after the irrigation. DNA-based analysis was performed by qPCR and amplicon sequencing of 16S rRNA gene.

Results: Spiking of wastewater increased the relative abundance of sulfonamide resistance (sul1), class 1 integron-integrase (intI1) genes, and plasmids belonging to IncP-1 and pSK1. The distribution of ARGs and MGEs showed no differences due to the water or soil type, but a significant separation due to the spiking. Specifically, soil microcosms irrigated with unspiked wastewater were associated with trimethoprim and erythromycin resistance genes, which significantly and positively correlated with inc18 and pI258 plasmids, typically hosted by Gram-positive bacteria. Soil microcosms irrigated with spiked wastewater were associated with IncP-1 and pSK1 plasmids, as well as intI1 and sul1 genes, which were significantly and positively inter-correlated. Analysis of the soil microbial community showed that only the soil type and sampling date determined the soil microbial composition. Among the 15 most abundant ASVs, five different members were significantly affected by the soil type while only a member affiliated to Pseudomonas was significantly affected by the spiking.

Implications: Further exogenous plasmid capturing experiments together with chemical data on antibiotic and biocide accumulation in soil will contribute to better understanding the role of plasmids, wastewater borne pollutants and associated microbiome on the spread of ARGs.

Author: Kornelia Smalla, Julius Kuehn Institut, Germany

Co-Author: Gallego, Sara; Soufi, Leila; Kampouris, Ioannis; Lüneberg, Kathia; Heyde, Benjamin; Siebe, Christina; Siemens, Jan; Grohmann, Elisabeth

Back to the Program

Session B

Abstract 34

Poster Board number: 85

Whole-genome analysis of ESBL-producing Escherichia coli and carbapenemase-producing Enterobacteriaceae in sewage collected at manholes

Background and Aim: The emergence of multi-drug resistant bacteria, especially those that can confer resistance to clinically significant antibiotics, largely limits treatment options for bacterial infections. We hypothesize that sewage, taken from manholes around the city, contains bacteria shed from a specific community and could provide accurate and quick information to understand the antibiotic resistance burden of the corresponding community. This study aimed to clarify the occurrence and characteristics of extended spectrum beta-lactamase-producing Escherichia coli (ESBL-EC) and carbapenemase-producing Enterobacteriaceae (CPE) in manholes across city A in Japan.

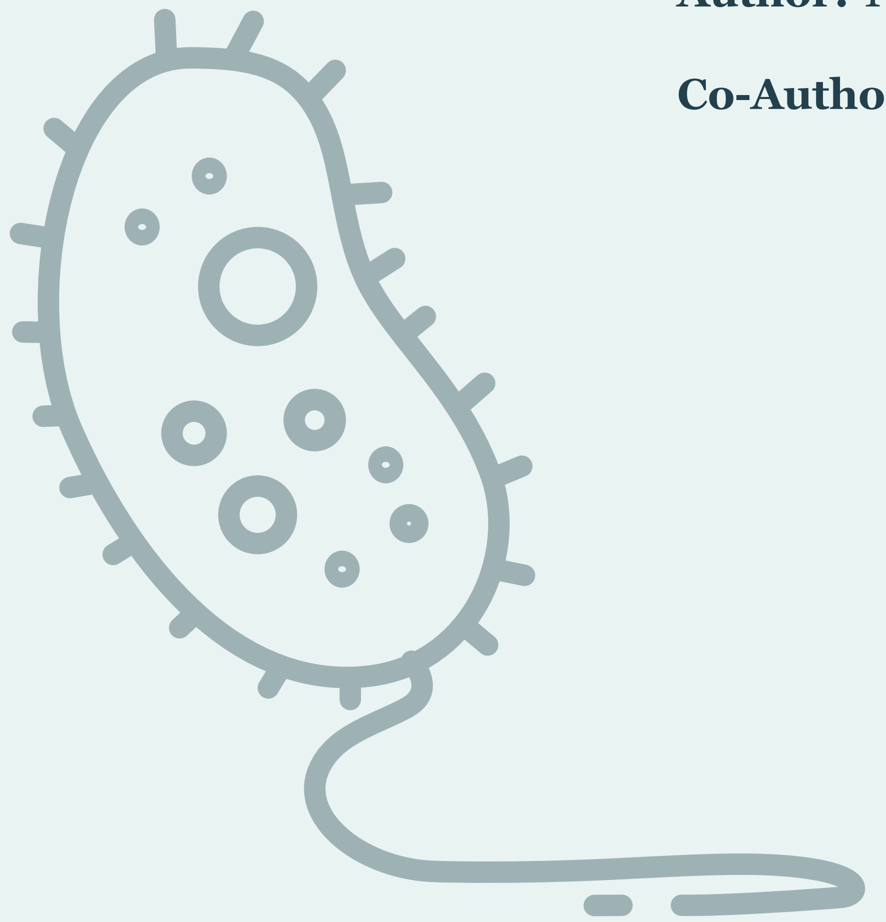
Method: Fifty-eight grab sewage samples were collected from 33 manholes during January 2022 to March 2023. Appropriately diluted samples were incubated on chromID ESBL and chromID CARBA to screen ESBL-EC and CPE, respectively. In total, 31 ESBL-EC and 12 CPE colonies were isolated and then subjected to short-read whole-genome sequencing (WGS).

Results: Based on the WGS of 31 ESBL-EC, sequence type (ST) ST38 (38.7%, 12/31) and ST131 (29.0%, 9/31) were the most prevalent. blaCTX-M-14 (38.7%, 12/31) was the most abundant ESBL gene, followed by blaCTX-M-27 (29.0%, 9/31), and blaCTX-M-15 (12.9%, 4/31). Twelve CPE isolates belonged to 5 species, including E. coli (n = 1), Enterobacter kobei (n = 3), Klebsiella quasipneumoniae subsp. similipneumoniae (n = 1), Klebsiella michiganensis (n = 2), and Raoultella ornithinolytica (n = 5). The most prevalent carbapenemase gene was blaGES-24 (58.3%, 7/12), followed by blaGES-4 (33.3%, 4/12), and blaNDM-5 (8.3%, 1/12). Our results were consistent with previous research that blaCTX-M-27 and blaGES-24 were frequently detected in Japan.

Implications: ESBL-EC were detected at all manholes, indicating their circulation in populations over the city and the risk of acquisition of community-onset infections. The higher relative abundance of blaGES and blaCTX-M genes in sewage provided important information for the empirical antimicrobial therapy towards ESBL-EC and CPE in clinics in Japan.

Author: Yu Tang, Kyoto University, Japan

Co-Authors: Gomi, Ryota; Nishimura, Fumitake; Ihara, Masaru



Session B

Abstract 131

Poster Board number: 86

Flash Poster Presentation: S11 - Tuesday, May 28 - 13:45-15:30

Genomic Analysis to Determine the Fate of Antimicrobial Resistant Escherichia coli in Soil Amended with Litter from Antibiotic-Fed Broiler Chickens

Background: Escherichia coli is used as an indicator bacterium of fecal contamination. Antimicrobial resistant and pathogenic E. coli strains have been reported in poultry litter. Objective: We applied a genomic approach to examine the persistence of antibiotic resistant E. coli in soils.

Method: Litters of 35-days old broilers fed a basal diet and basal diet with chlortetracycline, monensin, narasin or virginiamycin were applied to an agricultural field which was then sampled periodically to isolate and characterize E. coli from August to March. Based on their antimicrobial susceptibility profile, 30 E. coli isolates were genome sequenced and analyzed for antimicrobial resistance (ARGs) and virulence (VGs) genes. Phylogenetic tree was built, and genomes were compared.

Results: All 30 isolates belonged to six serotypes (10 O82:H8, 9 O8:H19, 4 O15:H45, 4 O1:H45, 2 O23:H16 and 1 O43:H34) and same serotypes were clonal in the same treatment over the time. Most isolates harbored IncF, IncB/O/K/Z, IncX1 and IncP plasmids. Two isolates of serotype O15:H45 at the 1st and 7th sampling times from the chlortetracycline treatment harbored the phage-like pO111. About 18 specific and 47 ubiquitous ARGs were detected with tetracycline resistance (tetAD), and β-lactamase (CMY-2 and TEM-1) genes being more prevalent in isolates at different months from soil amended with the litter of antimicrobial-fed birds than those from the control soil. Two (1st and 4th sampling times) isolates of serotype O23:H16 from soil treated with the litter of chlortetracycline-fed birds carried the aminoglycoside (aadA15) and trimethoprim resistance (dfrA1) genes and the several VGs were detected. Genetic relationships were found between isolates at different sampling times, indicating their persistence in soil.

Implication: The results of this study confirmed that multiple-antibiotic-resistant and potentially pathogenic E. coli can survive in soil fertilized with litter for months, highlighting, the necessity to develop litter management strategies.

Author: Paul Waliaula, University of Guelph, Canada

Co-Author: Islam, Rashedul; Yin, Xianhua; Lepp, Dion; Laing, Chad; Kiarie, Elijah; Diarra, Moussa Sory

Back to the Program

Session B

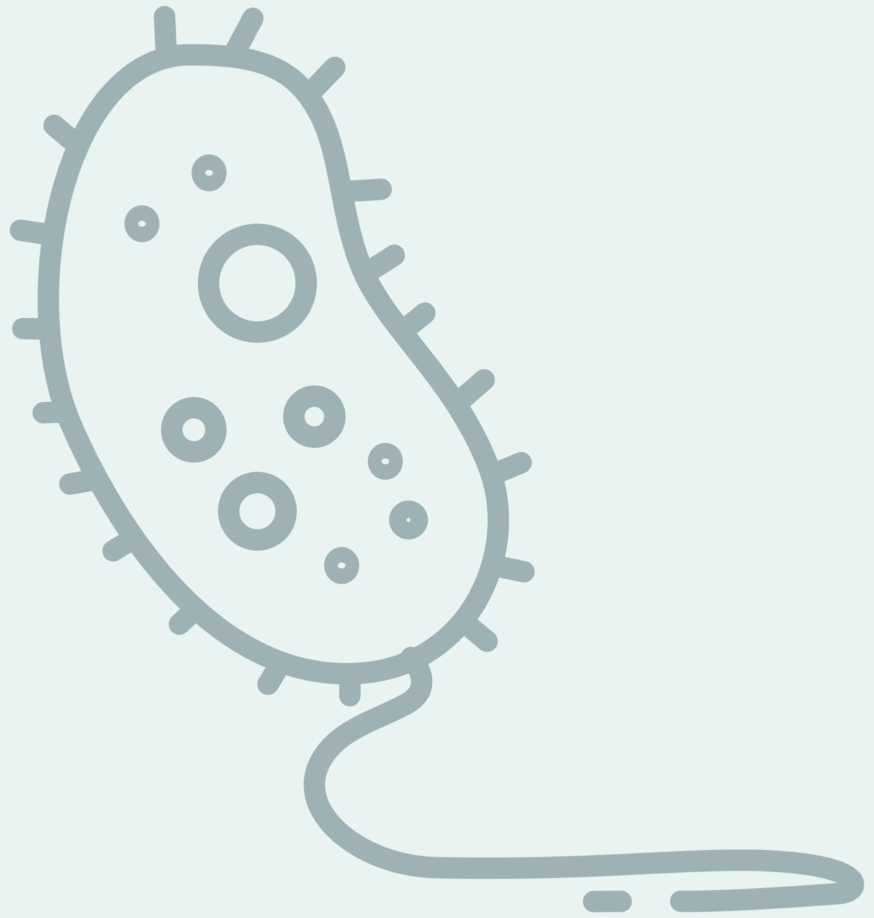
Abstract 296

Poster Board number: 91

An Asian Perspective on Antibiotics Contamination in Farm Soil Influencing Antibiotic Resistance Circulation in the Environment

Antimicrobial resistance or AMR is generally caused by unabated use of antimicrobials, mostly antibiotics, in the treatment of human and animal infections. Antibiotic resistance is a worldwide concern that involves the acquisition of resistance genes by significant bacteria in clinical settings. Most investigations on AMR are on human and animal health, whereas studies on AMR in the environment are limited. Many countries in Asia are densely populated with high food demand and production that require antibiotics use such as in animal farms. Asia is thus regarded as a potential hotspot for the unrestrained spread of antibiotic resistant bacteria. Animal farm soil is a component of the environment that contributes to the circulation of antibiotic resistant bacteria and resistance genes affecting human, animal, and plant health. Also, farm soils are often exposed to a variety of bioactive environmental pollutants that may co-select resistance genes and exacerbate the spread AMR. This paper examines and summarizes the burden of AMR in Asia, fate and transport of antibiotics from farm soil to the wider environment, and evidence of farm soil as a least-known but fundamental link in AMR transmission. These insights will contribute to the improvement of strategies and national action plans to combat the health risks of AMR in the region and globally.

Author: Maria Pythias Espino, University of the Philippines, Philippines



Session B

Abstract 299

Poster Board number: 92

Effect of ozonation of municipal wastewater on the reduction of antimicrobial resistance genes (ARG)

Background and Aim: Wastewater treatment plants (WWTPs) are recognized as hotspots for antimicrobial resistance. A final disinfection of the effluent, typically with UV or ozone, is often in place to reduce the bacterial load released to the receiving aquatic environment. However, its impact on ARGs is not well documented. The study aimed to evaluate the effects of ozone disinfection of a WWTP effluent applied at full-scale on selected ARGs and the interdependence with various physicochemical parameters of the water matrix.

Method: The study was conducted over 13 weeks in a WWTP located south of Montreal (Québec) and composed of grit removal, primary settling, activated sludge, settling and post-ozonation. Samples were collected weekly at the influent of the WWTP, the influent/effluent of the ozone process. Physico-chemical analysis were conducted on all water samples: pH, conductivity, chemical oxygen demand, total suspended solids, alkalinity, nitrite, sulphide, and metals (iron, zinc, copper, cadmium, cobalt and aluminium). Ozone concentration, flowrate and biochemical oxygen demand were collected from the plant. The abundance of 22 ARGs are analyzed using quantitative real-time PCR, including bla-TEM, bla-VIM, qnrA, tetM, Sul1 and vanA.

Findings: A significant difference in physico-chemical parameters was evidently observed during primary and secondary treatment but not during tertiary treatment using ozone. A slight increase in iron concentration was noted in effluents, while zinc and copper levels remained low. The quantification of ARGs in the 54 water and sludge samples are currently being analysed and will be completed by February 2024.

Implications: Results will help understand how the effectiveness of ARG elimination by ozonation could be influenced by physico-chemical parameters, helping to optimize the process and the future design of tertiary treatment using ozone. Furthermore, the co-selection of certain genes depending on the concentrations of heavy metals in water and sludge sample will be studied.

Author: Émilie Bédard, Polytechnique Montréal, Canada

Co-Author: Harrabi, Malek; Leal, Helena; Dorner, Sarah; Barbeau, Benoit

Back to the Program

Session B

Abstract 295

Poster Board number: 93

Flash Poster Presentation: S20 - Thursday, May 30 - 13:45-15:30

The ARISE project: Pioneering wastewater-based AMR surveillance in alignment with upcoming EU regulatory changes

In response to the growing challenges of antimicrobial resistance (AMR), the ARISE project emerges as a crucial two-year initiative, fostering a multi-sector collaboration among universities, industry partners, and key stakeholders. This project is strategically aligned with the upcoming revision of the European Urban Wastewater Treatment Directive (91/271/EEC), which mandates the implementation of AMR monitoring in wastewater systems to safeguard human and environmental health. The primary goal of ARISE is to establish a robust methodological foundation and advance best-practice strategies for routine AMR wastewater surveillance. This involves a comprehensive evaluation of key molecular analysis methods for detecting antibiotic resistance genes (ARGs), optimizing the entire workflow from sampling to biostatistical data analysis, and exploring innovative normalization approaches for ARG results like faecal source tracking and apportionment. Techniques such as qPCR, dPCR, array-based HT-qPCR, and shotgun-metagenomics are systematically compared and evaluated under standardized laboratory conditions and in targeted case studies, along with a cutting-edge sequencing method for targeted enrichment and analysis of >14,000 AMR markers. This novel and quantitative NGS approach, pre-evaluated on faecal impacted water and sewage samples, demonstrated sensitivity comparable to tested ARG-qPCR assays while exceeding shotgun-metagenomics in marker recovery. Furthermore, ARISE uniquely juxtaposes molecular methods with advanced phenotypic AMR assessment, providing comprehensive information on their comparability and effectiveness. This holistic and multi-dimensional approach aims to establish a solid knowledge base, vital for implementing an effective, future-oriented wastewater-based AMR surveillance system, as envisioned by the EU. The project also includes a social acceptance study to ensure methodologies and data outputs align with the needs and expectations of public health experts, clinicians, and other stakeholders. Overall, the outcomes of ARISE will be crucial for shaping future AMR wastewater surveillance and control strategies. Its applications extend beyond regulatory compliance, providing important insights for public health and environmental protection amidst the manifold AMR challenges.

Author: Claudia Kolm, Karl Landsteiner University of Health Sciences, Austria

Co-Authors: Vierheilig, Julia; Kreuzinger, Norbert; Zarfel, Gernot; Ströbele, Barbara; Finsterwald, Monika; Zepke, Georg; Martzy, Roland; Stelzer, Theresa; Weinberger, Johannes; Strauß, Reinhild; Lehner, Angela; Weber, Julia; Müller-Rechberger, Heide; Nykyforuk, Lia; Kirschner, Alexander; Farnleitner, Andreas

Session B

Abstract 315

Poster Board number: 94

Flash Poster Presentation: S20 - Thursday, May 30 - 13:45-15:30

Prevalence and Antimicrobial Resistance Profile of Bacterial Isolates from the Environment of Higher Education Institutions in Maputo, Mozambique

Background and Aim: The hands serve as the main body part through which individuals typically make initial contact with each other and the surrounding environment. There is growing concern regarding the transmission of pathogens, including drug-resistant microorganisms, through contact. This study aimed to evaluate microbial contamination on regularly sanitized objects and surfaces in two Higher Education Institutions (HEIs) in Maputo, Mozambique, and determine the antimicrobial sensitivity profile of the isolated bacteria.

Procedure/Method: In total, 129 swabs were collected from computer keyboards in student IT rooms, desks, and handrails in teaching units for Veterinary Sciences and Engineering and Technology courses. These swabs were taken before and after regular disinfection implemented for COVID-19 prevention. The swabs were cultured on modified nutrient agar, MacConkey, blood, and mannitol agar. Bacterial identification was done through standard bacteriological and biochemical tests. In total, 101 bacterial isolates were obtained and tested using the Kirby-Bauer disk diffusion method against nine antibiotics commonly used in both Human and Veterinary Medicine within Mozambique's national health system.

Findings/Results: The evaluated objects and surfaces exhibited high levels of microbial contamination particularly at Veterinary Sciences teaching units. The isolated bacteria included Streptococcus spp. (12%), Escherichia coli (11%), Micrococcus spp. (8%), Salmonella spp. (6%), Corynebacterium spp. (5%), Klebsiella spp. (5%), Proteus spp. (5%), along with other Gram-positive (15%) and Gram-negative (9%) bacilli. Overall findings indicated that 27.3% of isolates displayed high resistance to tetracycline, oxacillin, ceftazidime; while 35.2% demonstrated moderate resistance to piperacillin and polymyxin B. Around 37.8% of isolates were multidrug resistant, and 38.5% were sensitive to novobiocin, rifampin, azithromycin, and penicillin.

Implications/Applications: It is recommended to intensify cleaning and disinfection measures for objects and work surfaces in HEIs, practice frequent hand hygiene, and rotate disinfectants in order to prevent the spread of potentially harmful bacteria and drug-resistant microorganisms within the wider community.

Author: Cristiano Macuamule, Department of Animal and Public Health, Veterinary Faculty, University Eduardo Mondlane, Mozambique

Co-Author: Raice, Fátima

Back to the Program

Session B
Abstract 148

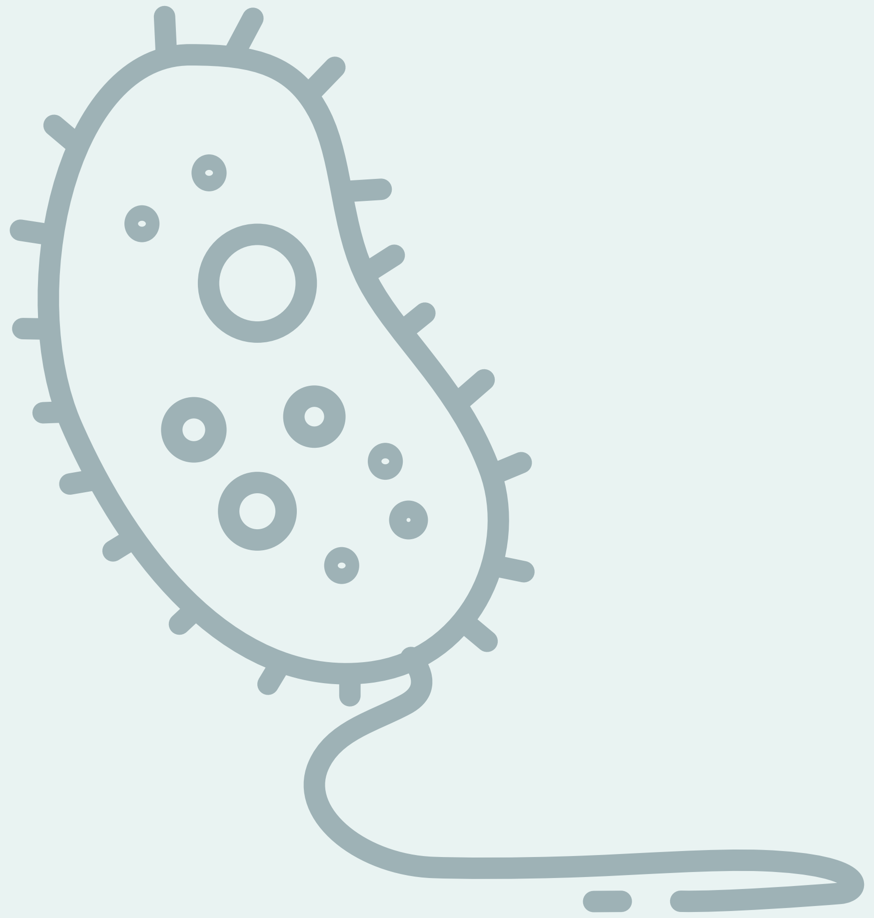
Poster Board number: 95

Flash Poster Presentation: S10 - Tuesday, May 28 - 13:45-15:30

THE IMPACT OF ANTIBIOTIC GROWTH PROMOTERS (AGPS) ON BROILER CHICKENS' HEALTH AND ENVIRONMENT IN KIBAHA TOWN COUNCIL – TANZANIA

Some poultry producers in Tanzania administer antibiotic growth promoters (AGPs) in the poultry production system to increase profits and efficiency of their birds by reducing keeping time and production costs, albeit at the expense of the environment and adverse health effects to human. Because of that, a cross-sectional survey was conducted at Kibaha Town to assess awareness and effects of using AGPs on both broiler chicken and environmental health, purposely for guaranteeing safe animal source foods (ASF) to consumers. A structured questionnaire was administered through face to face interview to 40 broiler chicken keepers, where qualitative and quantitative methods were used to collect data. Results showed that all respondents (n=40) had no diseased chickens in their houses, attributable to AGPs use, broiler boost and some hygienic practices. AGPs were administered by mixing with food (47.5%; n=19) or drinking water (52.5%; n=21) during the first week (45%; n=18), after first week (25%; n=10), after second week (12.5%; n=5) or continuously regardless of the withdrawal period (17.5%; n=7). The respondents administered AGPs themselves (62.5%; n=25) while 37.5% (n=15) had neither considered dosage nor the number of broilers kept in their poultry houses. Only 42.5% (n=17) of respondents knew about the negative effects of using AGPs whereas 57.5% (n=23) had no knowledge at all. Of these respondents 67.5% (n=27) sold the litter to vegetable growers and fish ponds owners as organic manure while 25% (n=10) randomly dumped it, only 7.5% (n=3) safely dumped it in shaded pits to avoid environmental contamination. It's concluded that broilers are fed AGPs, and their wastes are used as organic manure for agricultural fields and fish ponds production, posing risks to human, animals and environmental health. However, further studies on "One Health Approach" to analyze effects of AGPs use in the shared environment are currently needed.

Author: Damas Theobald Msaki, Kibaha Education Centre, Tanzania



Session B
Abstract 314

Poster Board number: 96

Reduced antibiotic resistome risks in pig manure after new FDA rule restricting antibiotic use

Background and Aim: To combat with the growing antibiotic resistance crisis, the US Food and Drug Administration (FDA) began to enforce the Veterinary Feed Directive (VFD) in January 2017 and outlawed the use of medically important antibiotics for growth promotion. We evaluated the efficacy and impact of the new policy on the profile and risks of antibiotic resistance genes (ARGs) in manures from pig farms.

Procedure/Method: We collected pig manure pit samples from finishing farms in 2008 and in 2018, and evaluated the dispersal, transfer and health risks of ARGs in the manure pit samples using the functional resistance enrichment and long-read sequencing method.

Findings/Results: We observed a significant drop in the resistome abundance in pig manures after the VFD implementation. Notably, abundances of ARGs corresponding to the affected antibiotics in the VFD, such as beta-lactams, tetracyclines and aminoglycosides declined by 33–57%. The overall resistome mobility also declined, and the profile of mobile genetic elements mediating horizontal gene transfer of ARGs significantly shifted. There was no significant difference in bacterial profile before and after the VFD implementation, while the composition of antibiotic resistant bacteria was significantly different, suggesting that the new policy affected ARG hosts. Although the VFD enforcement showed promise in mitigating manure risks, there were still a considerable amount of mobile ARGs carried by pathogens and their close relatives.

Implications/Applications: Our results suggest that the new VFD policy mitigates some resistome risks in pig manures but it is yet not sufficient, requiring more work and stewardship practices.

Author: Xun Qian, Northwest A&F University, China

Co-Author: Tiedje, James; Rozeboom, Dale; May, Gerald

Back to the Program

Session B
Abstract 297

Poster Board number: 97

Collection of sales and use data of veterinary antimicrobial products in Germany and the European Union

Background and Aim: With the new European veterinary legislation (Regulation (EU) 2019/6; NVR), harmonised regulations on veterinary medicinal products (VMPs) apply from January 2022. The new legislation covers not only rules on the authorisation and marketing of VMPs, but also measures to combat antimicrobial resistance (AMR). These include, among others, the collection of data on sales and use of antimicrobials in veterinary medicine. In Germany, the monitoring of sales volumes of veterinary anitbiotics was already implemented in 2010.

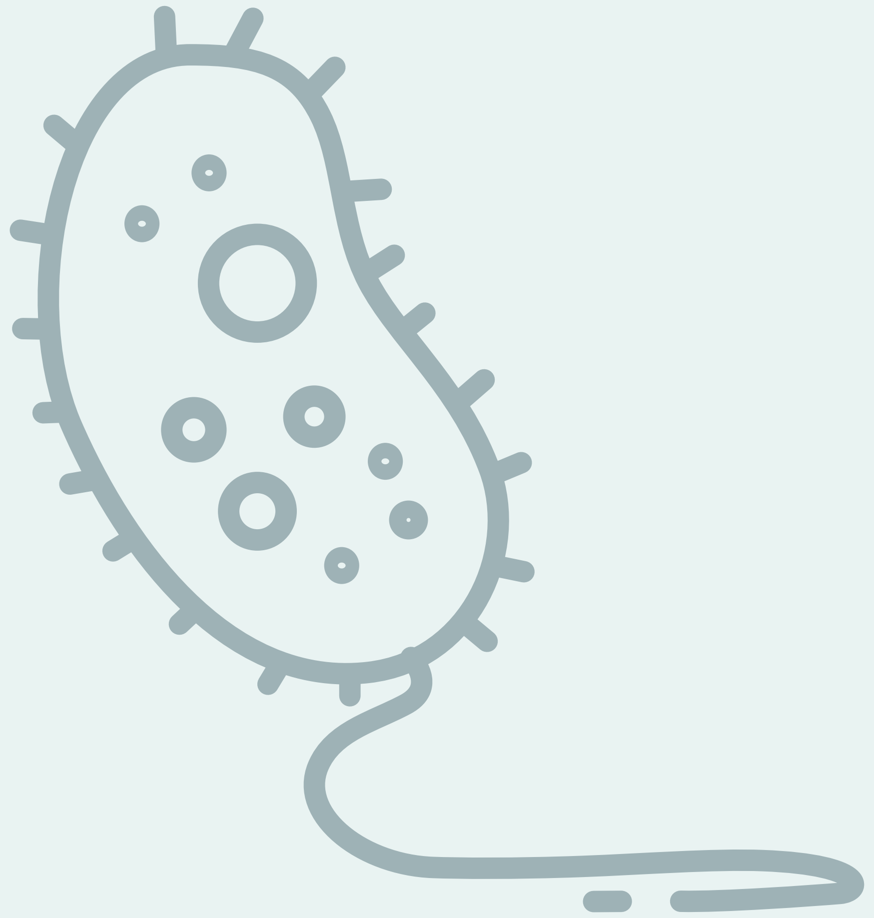
Procedure/Method: According to the NVR, data on veterinary antimicrobials sold in 2023 will have to be reported to the European Medicines Agency (EMA) in 2024. The collection of use data will be implemented stepwise. First, data on the use of antimicrobials in cattle, pigs and poultry must be reported to the EMA in 2024. Next, data on the use of antimicrobials in other food-producing animal species, including horses, have to be reported in 2027. Finally, data on the use of antimicrobials in dogs, cats and fur animals (minks and foxes) have to be submitted to the EMA in 2030.

Findings/Results: Sales data of antibiotic VMPs from 2022, still collected according to national legislation, were published by BVL and by EMA in November 2023. The first analyses of the use data will be available in autumn 2024. Meanwhile, the implementation of the next stages of use data is being planned and prepared.

Implications/Applications: Overall, it remains a challenging task for all stakeholders to implement the NVR. However, data on antimicrobial consumption in veterinary medicine are essential for analysing of the current situation and for evaluating the measures taken to combat AMR. With the NVR, harmonised and standardised data on sales and use data of antimicrobials in the European Member states will be available.

Author: Svenja Sander, Federal Office of COnsumer Protection and Food Safetys, Germany

Co-Authors: Klabunde-Negatsch, Alexandra; Siller, Paul; Heberer, Thomas



Session B
Abstract 368

Poster Board number: 98

Flash Poster Presentation: S16 - Thursdat May 30 - 8:30-10:15

Improving the DARTE-QM Monitoring Platform for a One-Health Surveillance Approach

Background and Aim: In developing an efficient method for monitoring antimicrobial resistance (AMR) genes in environmental ecosystems, the Diversity of Antimicrobial Resistance genes and Transfer Elements-Quantitative Monitoring (DARTE-QM) platform was developed. Efforts are now underway to enhance the initial DARTE-QM platform by refining AMR targets across a One Health spectrum, thus improving its applicability to risk assessment and monitoring of mitigation effectiveness. The study aimed to evaluate the diversity of AMR targets from sequenced human and animal pathogens that could be identified using the current primer set.

Procedure/Method: The designed DARTE-QM primers were screened for the prediction of their AMR targets to all known variants encompassed by the Resistomes and Variants collection of the Comprehensive Antibiotic Resistance Database (CARD, version 4.0.2). This screening employed the BLAST algorithm (version 2.14.0) with a word size parameter set to 7 for the initial match and a minimum of 90% nucleotide identity to primers.

Findings/Results: In a comparative analysis against the CARD resistomes database, the DARTE-QM primers captured 39% (2035/5221) of the antibiotic resistance genes (ARGs) with curated antibiotic resistance ontology (ARO) entries. Primer coverage for ARGs impacting major drug classes in the database included: aminoglycosides (59%, 114/193), chloramphenicol (40%, 19/47), colistin (48%, 52/108), fosfomycin (59%, 20/34), macrolide-lincosamide-streptogramin (50%, 43/85), quinolones (45%, 60/132), tetracycline (49%, 37/75), and trimethoprim (51%, 30/59). Notably, 33% (241/738) of DARTE-QM primers targeted beta-lactamases, capturing 42% (1535/3653) of the curated diversity of beta-lactamases, which dominate the CARD database (70%, 3653/5221). The DARTE-QM primers, designed to target 662 ARGs, are predicted to capture diverse variants within resistome families and subclasses due to sequence redundancy among ARG homologs. This showcases the primer set's versatility and highlights potential areas for improvement.

Implications/Applications: The project contributes significantly to global initiatives combating AMR, enhancing ARG surveillance, and fostering informed decision-making in the battle against AMR.

Author: Daniel Gyamfi Amoako, University of Guelph, Canada

Co-Author: Alcock, Brian; McArthur, Andrew; Ricker, Nicole

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 256

Poster Board number: 100

Uncovering the contribution of viruses to antibiotic resistance spreading in paddy soils with manure application

Antibiotic resistance is an urgent threat to global health, causing many antibiotic-resistant infections and deaths. The viruses infect bacteria and act as vectors for transfer of antibiotic resistance genes (ARGs) via transduction, increasing the ecological risk of ARGs in environments. However, the contribution of viruses to ARGs dissemination in soil ecosystem is poorly explored. Here, we investigated the ARGs transfer between viruses and bacteria in the organic and inorganic fertilizers-applied paddy soils by metagenomic and viromic sequencing without amplification bias. Soil viromes showed that the families Siphoviridae, Podoviridae and Myoviridae accounted for > 71% of the identifiable viruses. Fertilization facilitated the restructure of the phage-bacterial ecological network and manure application significantly increased the abundance of genes encoding stress-associated functions and sulfur/phosphorus cycling. The relative abundance of ARGs in viral DNA fraction was 1.30- to 1.58-fold higher than that in bacteria, and quinolone resistance genes were significantly enriched in the manure-applied soil virome. Manure application significantly increased the numbers of shared genes between soil virome and soil metagenome and also increased the diversity of host bacteria for phage-associated ARGs. These results indicate that soil phages is a potential ARGs reservoir, playing a vital role in horizontal transfer of ARGs, and manure application can promote the transfer and spread of ARGs mediated by phages in soil, exasperating the risk of ARGs spreading in soil.

Author: Xin-Li An, Institute of Urban Environment, Chinese Academy of Sciences, China

Co-Authors: Wang, Jia-Ying; Su, Jian-Qiang

Session B

Abstract 8

Poster Board number: 101

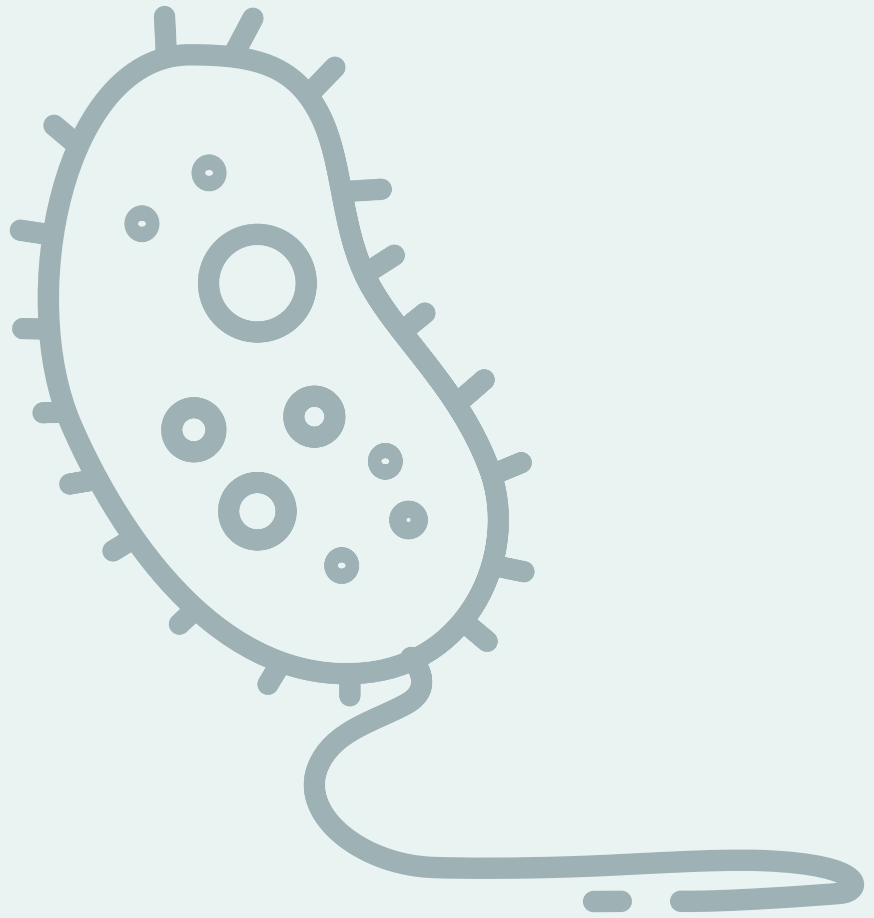
Flash Poster Presentation: S20 - Thursday, May 30 - 13:45-15:30

Strain-level diversity in sulfonamide biodegradation: adaptation of Paenarthrobacter to sulfonamides

The widespread occurrence of sulfonamides raises significant concerns about the evolution and spread of antibiotic resistance genes. Biodegradation represents not only a resistance mechanism but also a clean-up strategy. Dynamic and diverse environments shaped both the structure and function of microbial communities, resulting in the distinct cellular function of individual sulfonamide-catabolic strains. Here, we present Paenarthrobacter from different origins that demonstrated diverse growth patterns and sulfonamide-degrading abilities. Generally, the degradation performance was largely associated with sadA gene copies and also relied on its genotype. Based on a genome-centric survey of sad genes, an independent mobilization of transposon-borne genes between chromosome to plasmid was observed. Noteworthy, insertions of multiple sadA genes could greatly enhance sulfonamide-degrading performance. Moreover, the sad gene cluster and sadA transposable element showed phylogenetic conservation currently, being identified only in two genera of Paenarthrobacter (Micrococcaceae) and Microbacterium (Microbacteriaceae). Meanwhile, Paenarthrobacter exhibited a high capacity for genome editing to adapt to the specific environmental niche, opening up new opportunities for bioremediation applications.

Author: Yue Huang, The University of Hong Kong, Hong Kong

Co-Author: Zhang, Tong



Back to the Program

Session B

Abstract 77

Poster Board number: 103

Experiences from wastewater treatment trials using ozone and UV-irradiation to mitigate AMR dissemination from AMR hotspots.

Background and Aim: Today's urban wastewater treatment plants (UWWTPs) are incapable to fully remove ARBs, ARG and antibiotic resistance driving substances from wastewater, contributing to the release of AMR into the environment, potentially entailing health hazards. Advanced wastewater treatment locally at AMR sources, such as hospitals, and centralized at WWTPs could provide possible intervening solutions. However, currently knowledge about the effectiveness, large-scale application, long-term operational stability, and economic conditions, is scarce, despite being urgently needed by stakeholders.

In the framework of the Norwegian CORNELIA project, the objective was to develop and demonstrate compact pilot-scale treatment technologies using ozone and UV-irradiation, to remove ARBs and ARG in wastewater effluents from hospitals and UWWTPs. Results and experiences from the test campaigns will be presented.

Procedure/Method: Wastewater effluents were treated by two pilot systems using ozone and UV either alone or in series. Different ozone and UV doses were assessed. Samples were collected consecutively, and bacteria were analyzed by culturing and molecular approaches.

Findings/Results: The treatment of WWTP effluents with ozone at ca. 2-5 g O3/m3 and UV-irradiation at 2000 J /L alone led to full inactivation of bacteria measured by colony-forming units (CFU) growth on blood-agar, equal to 4-5 log10 and 3-4 log10 reduction. qPCR results indicate that the DNA in treated samples was unreadable damaged. UV-irradiation of ozonated water only insignificantly increase the removal effectiveness achieved by ozone alone. In samples receiving lower ozone doses (yet not in those >13 g O3/m3), bacteria belonging to various families regrow within 5 days of re-cultivation, indicating that certain bacteria were able to persist or repair the damages. At EDAR, we will also present results received from the treatment of hospital wastewater effluents.

Implications/Applications: The results imply a strong short-term hygienization potential by ozonation and UV-irradiation. The observed reactivation potential necessitates further investigations.

Author: Carsten Ulrich Schwermer, Norwegian institute for water research (NIVA), Norway

Co-Authors: Ingebretsen, Trond; Olafssøn, Halfdan; Osinska, Adriana D.; Holmstad, Rune; Due, Nina; Wasteson, Yngvild

Session B

Abstract 410

Poster Board number: 104

Flash Poster Presentation: S9 - Tuesday, May 28 - 11:00-12:30

Spatial differences in the human gut microbiota selects distinct antimicrobial resistance genes in a simulated in vitro reactor

Background and Aim: While most environmental studies determine the level of human exposure to antimicrobial resistance genes (ARGs), true risk quantification requires the combination of the exposure with the host system response. A possible response could be the level of dissemination and persistence of environmental ARGs in the human gut microbiota, which maybe influenced by the pH differences in the colonic compartments. Therefore, we aim to study the spatial differences in the ARG abundance using an in vitro gut reactor.

Procedure/Method: Four 10-day runs were carried out with five completely mixed semi-continuously flowing reactors simulating digestion (Habib et.al.,2021). After the first two axenic reactors simulating gastric(pH=2) and small intestinal(pH=7.5) conditions, the last three reactors representing the ascending(pH=5.9), transverse(pH=6.3), and descending(pH=6.7) colon were inoculated with human feces. Community composition was assessed by 16S rRNA gene amplicon sequencing and 75 ARGs (10 antimicrobial classes) were quantified by high-throughput qPCR array (Resistomap).

Findings/Results: Over the 10 days of reactor operation, the total abundance of certain ARG classes (e.g.: against MLSB) increased in all colonic compartments while the abundance of certain other ARG classes (e.g.: against β-lactam) decreased. Towards the end of the runs as the microbial community neared steady-state, we observed differences in ARG abundance between colonic compartments. For example, the total abundance of ARGs against MLSB at the end of the runs were 0.180±0.012 (average of 4 runs±standard error), 0.197±0.005 and 0.217±0.012 ARG copies/16S rRNA gene copies, respectively in ascending, transverse, and descending colon reactors. The preliminary differences in relative ARG abundances across the colon compartments suggest variations in the host response in ARG dissemination.

Implications/Applications: Understanding the spatial variations in ARG abundances in colonic compartments and their steady state levels without environmental exposure/antimicrobial selection will be necessary to adequately parameterize the dose-response of environmental ARG dissemination to the human gut microbiome.

Author: Fathima Afsal, McGill University, Canada

Co-Author: Swarupha, Jothy; Frigon, Dominic; Kubow, Stan; Iskander, Michele; Gaisawat, Mohd Baasir; Lopez Castro, Juan Sebastian

Back to the Program

Session B

Abstract 255

Poster Board number: 105

First characterization of the prevalence and antimicrobial resistance profile of Salmonella in Quebec sheep flocks

Background and Aim: Salmonella is a major cause of foodborne disease of bacterial etiology in humans. The spread of antimicrobial resistance (AMR) Salmonella strains is a serious concern for both human and animal health. Food-producing animals play an important role in human salmonellosis. However, scientific studies characterizing the prevalence and the AMR profile of Salmonella in Quebec sheep production is lacking. This study aimed to estimate the prevalence of Salmonella-positive ovine flocks in Quebec, identify the predominant Salmonella serotypes, and characterize the AMR profile of the Salmonella strains isolated from these flocks.

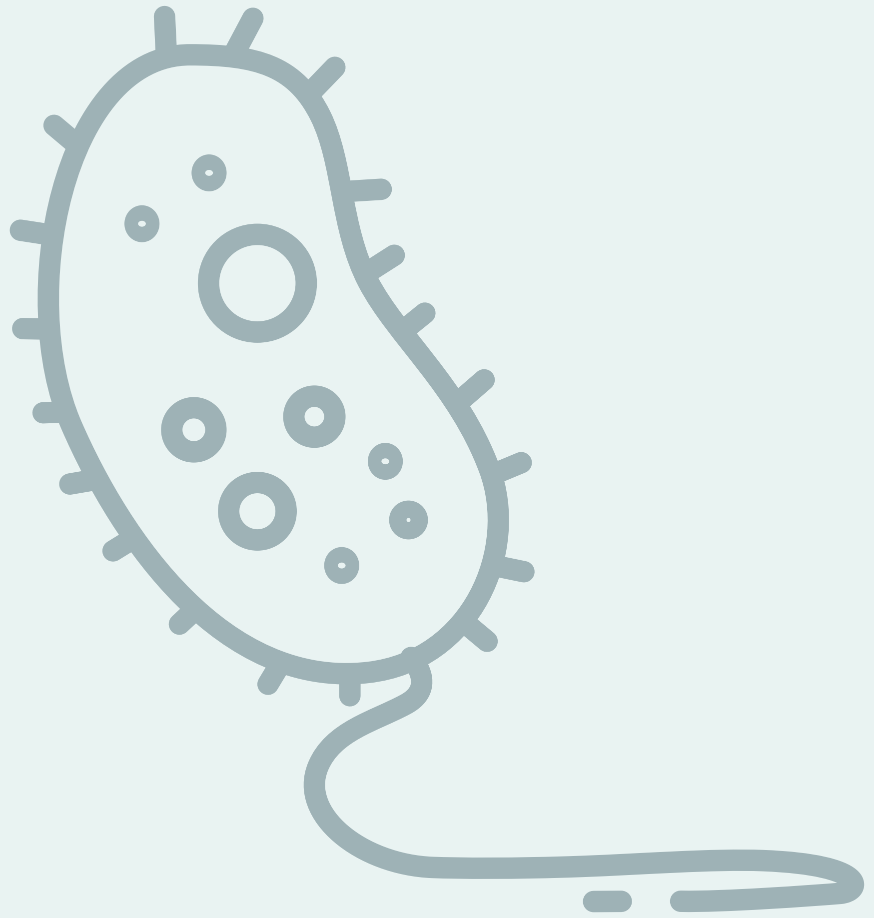
Procedure/Method: A total of 61 Quebec sheep farms were randomly selected and sampled. A fecal pool from 10 animals was collected per farm. Dilutions of each sample were plated in selective media to isolate Salmonella spp. The identification of Salmonella isolates was carried out using biochemical tests and PCR targeting the invA gene. Whole-genome sequencing (WGS) was used to predict Salmonella serotypes and AMR genes of 86 selected Salmonella strains. The sensitivity of these strains to 7 antimicrobials was also assessed by the disk diffusion test or by the broth microdilution assay.

Findings/Results: This study showed that 83.6% of Quebec sheep flocks are Salmonella carriers. Only Salmonella enterica subsp. diarizonae serotype 61:k:1,5,(7) was identified in the strain collection. Moreover, no AMR genes were detected by WGS in these strains, and all of them were phenotypically susceptible to the antimicrobials tested.

Implications/Applications: The present study was the first to identify the 61:k:1,5,(7) serotype in sheep farms of Quebec and highlight its exclusive prevalence. The antimicrobial susceptibility of all tested strains could be explained by the limited use of antimicrobials in the sheep farms. It is therefore important to determine the pattern of antimicrobial use on Quebec sheep farms, and to establish the genetic link between sheep and human serotypes.

Author: Schlasiva Cenatus, Université de Montréal, Canada

Co-Authors: Arsenault, Julie; Bernaquez, Isabelle; Bekal, Sadjia; Thériault, William P.; Rhouma, Mohamed



Session B

Abstract 250

Poster Board number: 106

Effect of Advanced-Treated Biosolids Application on AMR in Soil

Background and Aim: Land application of biosolids can be a sustainable way to reuse nutrients in wastewater treatment plant (WWTP) residuals as fertilizer. However, the majority of resistant bacteria entering WWTPs partition to the solids. Here, we carried out a field study to determine the effects of application of biosolids produced by thermal hydrolysis and anaerobic digestion on antibiotic resistance in soil.

Procedure/Method: A cornfield that had not been subject to manure or biosolids application in the last nine years was divided into three experimental plots with different soil types (USDA soil series: Acredale, Tomotley, and Bojac) plus a control plot with no biosolids application. Biosolids, soil, and surface runoff samples were collected before land application, after land application, and after corn harvest and subject to culturing of cefotaxime-resistant (CefR) and total Escherichia coli. Post-harvest soil samples were re-cultured after 2.5 weeks of incubation at 4°C and counted again after 6 additional weeks to test for cold-temperature survival of E. coli and carbapenem-resistant Enterobacteriaceae (CRE).

Findings/Results: Total and CefR E. coli numbers in soil were not affected by biosolids application. In the biosolids, total E. coli counts were not significantly different from the soil and CefR E. coli was not detected. Refrigeration did not affect total and CefR E. coli colony counts. However, refrigerating the soil samples and Petri dishes for several weeks yielded quantifiable CRE counts at a median concentration of 243 CFUs/gram of dry soil.

Implications/Applications: The findings provide insight into whether land application of advanced-treated biosolids presents concerns for release of resistant enteric pathogens to soil environments. Thermal hydrolysis is expected to produce an essentially sterile influent to the anaerobic digester, but the digester microbial community could still be a significant reservoir of resistant bacteria. Cold storage helped to recover resistant organisms of interest.

Author: Clayton Markham, Virginia Tech, United States

Co-Author: Maldonado Rivera, Gabriel; Maile-Moskowitz, Ayella; Nguyen, Loc; Amaral-Torres, Amber; Manthapuri, Vineeth; Byrne, Thomas; Goh, Ying-Xian; Fontaine, Ayanna; Hoffman, Kenneth; Richardson, April; Pruden, Amy

Back to the Program

Session B

Abstract 360

Poster Board number: 107

Subinhibitory biocide exposure is more likely to reduce than stimulate conjugal transfer of resistance plasmids

Background and Aim: Currently, there is evidence suggesting that exposure to diverse antibiotics, biocides, and other non-antibiotic compounds can stimulate conjugal transfer of the model resistance plasmid RP4. Yet, a systematic assessment across classes of biocides and plasmids is lacking. Therefore, we aimed to determine how various biocides influence conjugation across different plasmid incompatibility groups and donor/recipient combinations.

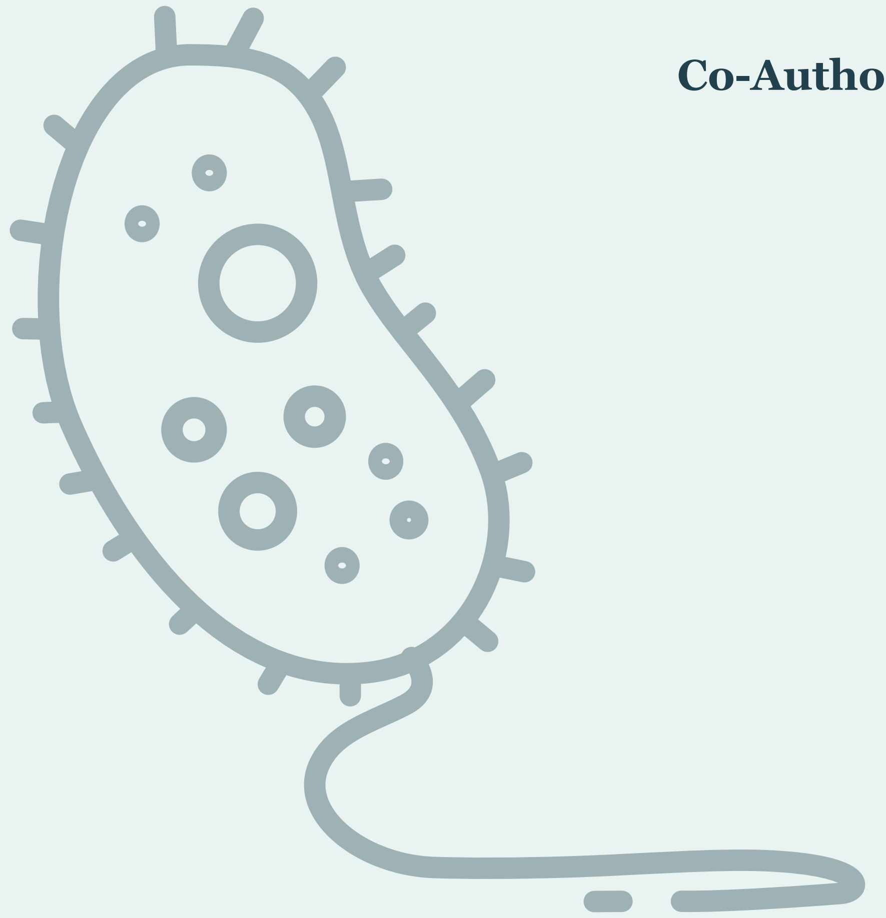
Procedure/Method: We conducted conjugation assays using conjugative plasmids RP4, R27, and R1 from the IncP-1α, IncHI1, and IncFII groups, respectively. Escherichia coli MG1655 or Pseudomonas putida KT2440 carried the plasmids, while E. coli MG1655 and P. putida KT2442 served as recipients at densities of approximately 1E+08 CFU/ml. Mating occurred in PBS to avoid growth bias, either supplemented with biocide at two concentrations (1% or 10% of MIC) or without (baseline). The biocides spanned a range of chemical classes, including metals, chlorophenols, biguanides, QACs, aromatic acids, triazoles, isothiazoles, parabens, phenols, chloramines, peroxy acids. The transconjugants, recipients, and donors were quantified using a Most-Probable-Number method.

Findings/Results: Across biocides and plasmids and mating pairs, stimulation of plasmid transfer was rare as it was limited to RP4 in E. coli and 4 out of 15 biocides. Even when stimulation was detected, it was modest (1.5 – 4 fold increase compared to baseline). In contrast, the transfer of R27 and R1 was commonly inhibited (6/6 and 4/6 biocides, respectively) and to a high degree (1.5 – 51.7 fold decrease compared to baseline). This plasmid dependent behavior suggests a connection with mechanisms governing plasmid transfer regulation. Experiments are underway to validate the generality of these findings.

Implications/Applications: While the current perception is that stimulation of plasmid transfer is commonplace, our study provides a more nuanced view by showing plasmid- and host-specific impact of biocide exposure. We expect that this insight inform novel, plasmid-class-specific risk assessments of chemicals.

Author: Zhiming He, Technical University of Denmark (DTU Bioengineering - Department of Biotechnology and Biomedicine), Denmark

Co-Authors: Dechesne, Arnaud; Smets, Barth; Jelsbak, Lars



Session B

Abstract 286

Poster Board number: 108

Flash Poster Presentation: S9 - Tuesday, May 28 - 11:00-12:30

Elucidating the Transport and Fate of Antibiotic Resistance Genes from Fresh and Treated Cattle Manure in Freshwater Streams

Land application of animal manure is a prominent source of Antibiotic Resistance Genes (ARGs) to the environment Rainfall events can cause ARG-contaminated runoff from amended soils to reach nearby streams. Thus, it becomes paramount to understand the dynamics of manure-borne ARGs in surface waters to tackle the advance of antibiotic resistance in the environment. Here, we aim to describe the transport characteristics of ARGs from fresh and treated manure in freshwater streams. Manures were collected in a dairy farm and then injected along with a conservative tracer (i.e., NaCl) in four artificial streams (50 m long) supplied with groundwater (flow rate 2 L/sec). Upon reaching the steady-state determined by the tracer, we collected water samples (n=4) at five sampling sites downstream of the release point at each stream. Samples were then filtered in the laboratory, and the DNA was extracted. The concentration of two ARGs (tetQ and tetW) was determined using digital PCR. Linear regressions were then fitted to the molecular data and average first-order removal rates (k; m-1) were obtained for each target and each type of manure. Our results showed that ARGs from fresh manure were removed significantly faster in comparison with treated manure (ANOVA; $p < 0.05$). For instance, overall k for tetQ were -0.009 and 0.005 m-1 in fresh and treated manure, respectively. Further analysis of the samples, including metagenomics and removal of extracellular ARGs will be done to better understand the observed transport. Overall, our work contributes to the understanding of the dynamics of ARGs in the environment and will be a valuable resource to mitigate the impact of agricultural activities in the spread of resistance.

Author: Andrei Badilla-Aguilar, University of Notre Dame, United States

Co-Author: Ginn, Olivia; Vu, Kevin; Oceguera-Johnson, Bethany; Thrift-Cahall, Emma; Liddick, Mitchell; Tank, Jennifer; Bolster, Diogo; Bibby, Kyle

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 230

Poster Board number: 111

Quantitative microbial risk assessment for waterborne pathogens in a wastewater treatment plant and its receiving surface water body

Background and Aim: Access to safe water for drinking and domestic activities remains a challenge, forcing resource-constrained communities to use microbiologically polluted surface water for domestic purposes, posing a public health risk. This study quantified bacterial contamination and the potential health hazards that wastewater treatment plant (WWTP) workers and communities may face after exposure to waterborne pathogenic bacteria in a WWTP and its associated surface water.

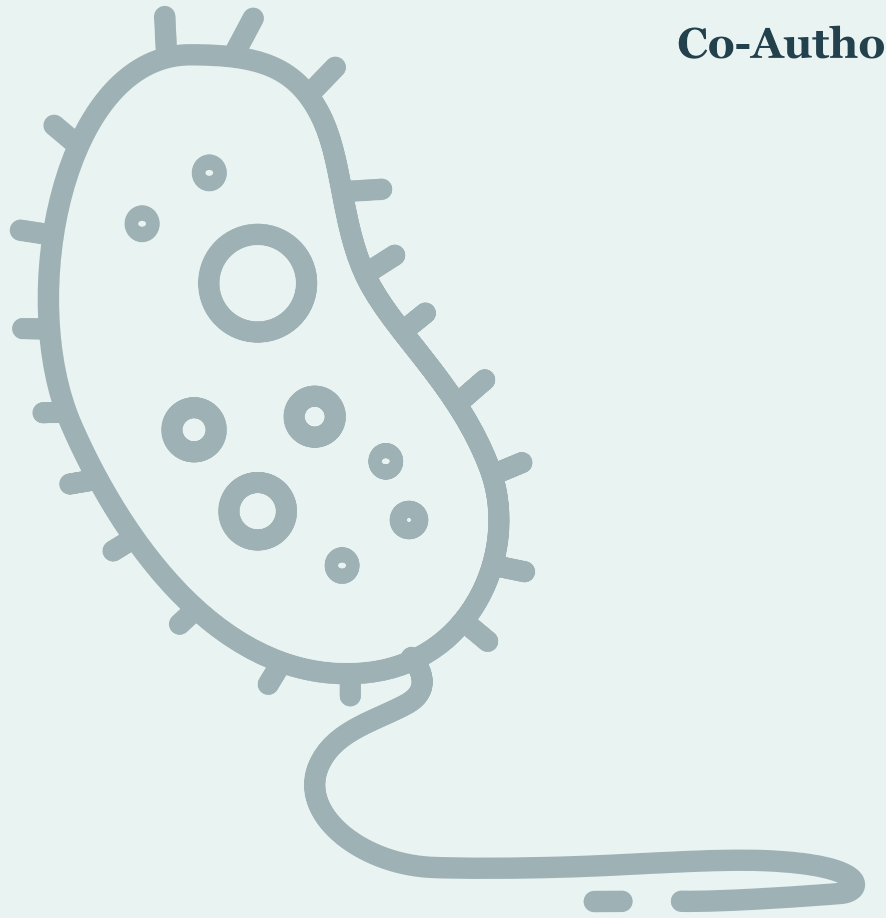
Procedure/Method: Escherichia coli and enterococci were quantified using the Colilert®-18 and Enterolert® Quanti-Tray® 2000 and definitively identified by real-time polymerase chain reaction targeting the uidA and tuf genes, respectively. An approximate beta-Poisson dose-response model was used to estimate the probability of infection (Pi) with pathogenic E. coli.

Findings/Results: Mean E. coli concentration ranged from 2.60E+ 02/100 mL to 4.84E+ 06/100 mL; enterococci ranged from 2.60E+ 02/100 mL to 3.19E+ 06/100 mL across all sampled sites. Of the 580 E. coli isolates, 89.1% were intestinal, and 7.6% were extraintestinal pathogenic strains. The 579 enterococci consisted of E. faecalis (50.4%), E. faecium (31.4%), E. casseliflavus (3.5%) and E. gallinarum (0.7%). The community health risk assessment revealed a greater health risk (Pi) from the ingestion of 1 mL of river water from upstream (range, 55.1–92.9%) than downstream (range, 26.8–65.3%) sites. The occupational risk of infection with pathogenic E. coli for workers resulting from a once-off unintentional consumption of 1 mL of water was 0% (effluent) and 23.8% (raw influent). Multiple weekly exposures of 1 mL over a year could result in a Pi of 1.2 and 100% for the effluent and influent, respectively.

Implications/Applications: Our findings revealed a potentially elevated risk of infection for WWTP workers and communities that use surface water upstream and downstream of the investigated WWTP. There is a need for constant monitoring of pollution levels in surface water sources used by vulnerable communities.

Author: Sabiha Essac, University of KwaZulu Natal, South Africa

Co-Authors: Mbanga, Joshua; Abia Akebe, Luther King; Gyamfi Amoako, Daniel



Session B

Abstract 283

Poster Board number: 113

Impact of an obesogenic diet on the transmission of antibiotic resistance genes from bioaerosols to gut microbiota in mice

The widespread use of antibiotics influences our exposure to antibiotic resistance genes (ARGs) from the bacteria surrounding us. The ability of ARGs to be transmitted to the intestinal microbiota is less understood although recent studies reported that consuming a diet rich in fat and in sugar facilitates the transmission of ARGs to the gut microbiota in mice. The objective of this project is to determine if ARGs from bacteria sampled from agricultural air can be transmitted to the gut microbiota in mice and if an obesogenic diet rich in fat and in refined sugar (HFHS) but also containing a mix source of proteins (PM) that are more relevant to human consumption influences ARGs transmission. We performed a preliminary in vitro protocol in which we cultivated Enterococcus faecalis containing a known ARG to tetracycline isolated from conventional pig farms, in the presence of a feces solution from either a lean mice fed a chow or an obese mice fed a HFHS-PM diet for 4 weeks. These bacterial solutions were then cultivated in presence of different concentrations of tetracycline (1 µg/ml, 2 µg/ml or 16 µg/ml). After 72h, a greater bacterial growth in the HFHS-PM condition was observed in the presence of the antibiotic as compared to chow condition. These preliminary results confirm our hypothesis that a HFHS-PM diet creates a suitable environment in the gut microbiota for the transmission of ARGs between bacteria. We are now performing an in vivo protocol to confirm that ARGs are transmitted more efficiently within the intestinal microbiota of mice fed the obesogenic HFHS-PM diet as compared to that found in chow-fed mice. These results will allow us to better understand how exposure to ARGs from bioaerosols influences the resistome of the intestinal microbiota and if poor dietary habits make us more susceptible to ARG transmissions.

Author: ILARIA SCAPINO, Institut universitaire de cardiologie et de pneumologie de Québec - Université Laval, Canada

Co-Author: Marette, André; Duchaine, Caroline; Pilon, Geneviève; Topp, Edward; Turgeon, Nathalie; Lebeuf, Maria; Lemieux, Joanie

Back to the Program

Session B

Abstract 253

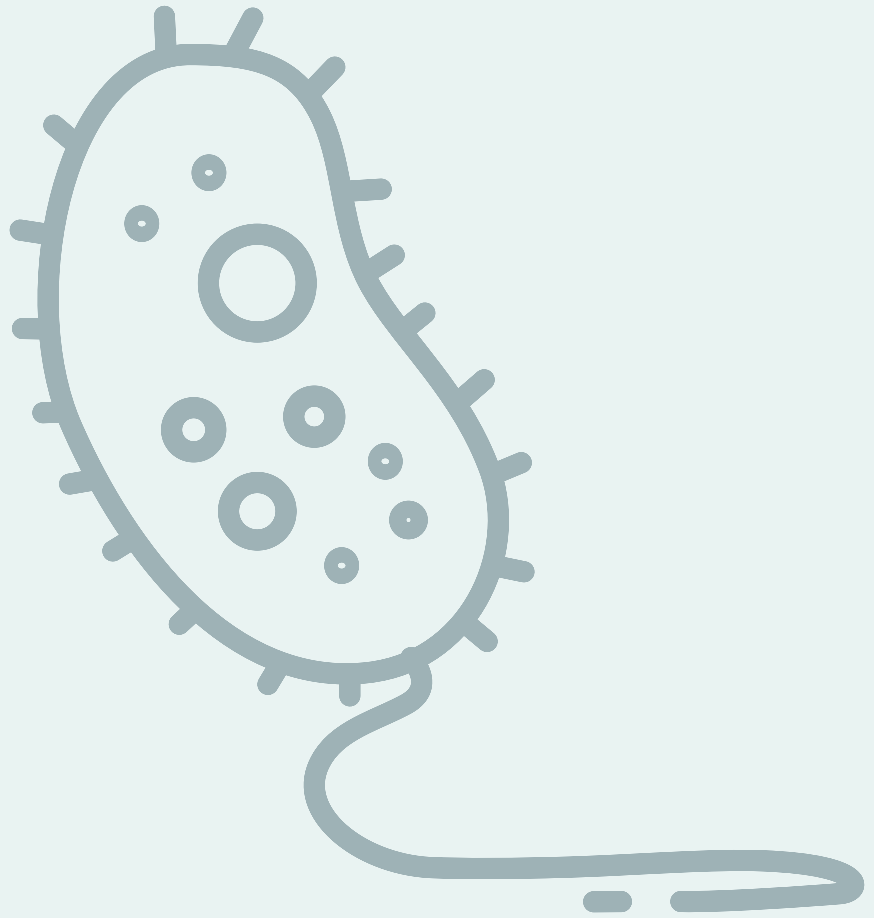
Poster Board number: 114

Flash Poster Presentation: S11 - Tuesday, May 28 - 13:45-15:30

Correction effect of Gram positive and Gram negative in extraction and sequencing process based on whole cell spike-in method

With the rapid development of sequencing technologies, higher demands are placed on the development of the microbial field. Standardization and quantification are the primary direction of metagenomic analysis, and a crucial aspect here is the conversion of relative abundance to absolute abundance. Absolute quantification can be used for risk assessment in various environments. And absolute abundance enables cross-sample comparisons, which can link various habitats, making microbial research more systematic and coherent. Additionally, absolute abundance can accurately reflect the dynamic changes in microbial composition over time and space. This study is divided into four parts. Part 1 involves the cultivation of two bacteria (one G+ and one G- bacteria) as spike-ins, the confirmation of pure culture bacteria through Nanopore sequencing and cell counting used both cultivation and flow cytometry methods. Next, the applicability of these two spike-ins in diverse conditions have been confirmed. In part 2, the accuracy of whole cell spike-ins is validated using mock community with known absolute abundances of each component. The correction of extraction and sequencing efficiency biases of G+ and G- bacteria used these two spike-ins have been assessed. Part 3 analyses eight type environment added with spike-ins for absolute abundance of microbials, pathogens, ARGs. Horizontal comparisons are conducted between samples within the same environment and across different environments. This study explores the correlations between important microbials and ARGs/MGEs in different environments, as well as variations in the carriage of ARGs by key microbials across different environments. In part 4, this study investigates the differences in the removal rates of various pathogens, ARGs, and ARGs carried by key microbials among five different wastewater treatment processes.

Author: Xiang Hui Shi



Session B

Abstract 364

Poster Board number: 115

Flash Poster Presentation: S9 - Tuesday, May 28 - 11:00-12:30

Exploring python scripts in Geographic Information Systems (GIS) to reveal distribution patterns of antimicrobial resistance in the environment

Background and Aim: Spatial analysis of antimicrobial resistance (AMR) in the environment is an understudied subject. The lack of continuous surveillance data for AMR-pollution in the environment has made it hard to produce statistically reliable spatial analyses. Using waste water samples from southern Sweden we aim to test automated python scripts in GIS to spatially relate relative abundance of antibiotic resistance genes (ARGs) to possible point sources in water catchment areas.

Procedure/Method: Via triangulation and interpolation, we connect AMR-occurrence with point-sources, mainly in the form of discharges from known polluters such as agriculture, wastewater treatment plants, and hospitals. For each location water samples from water treatment facilities were analyzed for normalised values of antibiotic resistance genes (ARGs). Automated python scripts in GIS are used to analyse the possible contributions from different types of point sources to the abundance of five different ARGs, where three of the ARGs are common in animal production and two are common among human pathogens.

Findings/Results: The five study locations located in Uppland, Sweden, revealed variance in land use, degree of urbanisation and a variance in ARG-levels. Preliminary results point to differences in distribution patterns for each location. We find that an automated approach compared to manual input made it possible to test many variables simultaneously. The method does have its limitations and with an increase in data volume, e.g. larger areas, machine-learning or AI-methods may need to be applied to better manage “big data”.

Implications/Applications: Applying automated scripts to test statistical significance of an array of distances may make analyses at a larger scale more relevant, may spatially-guide sampling efforts, and ultimately assist decision making in mitigation efforts.

Author: Patrick Spets, Södertörn University, Sweden

Co-Authors: Ebert, Karin; Dinnétz, Patrik

Back to the Program

Session B

Abstract 39

Poster Board number: 116

The fate of high-risk antibiotic resistance genes in secondary and tertiary municipal wastewater treatment plants

Background and Aim: Wastewater treatment plants (WWTPs) are important sources of antibiotic resistance genes (ARGs) in the environment. High-risk ARGs, which refer to those transferable ones enriched in human-related environments and accessible by pathogenic bacteria, pose a more direct and significant threat to human and animal health. Therefore, understanding the fate and expression of high-risk ARGs during wastewater treatment processes, are the prerequisites for effectively combating antibiotic resistance in the “One Health” framework.

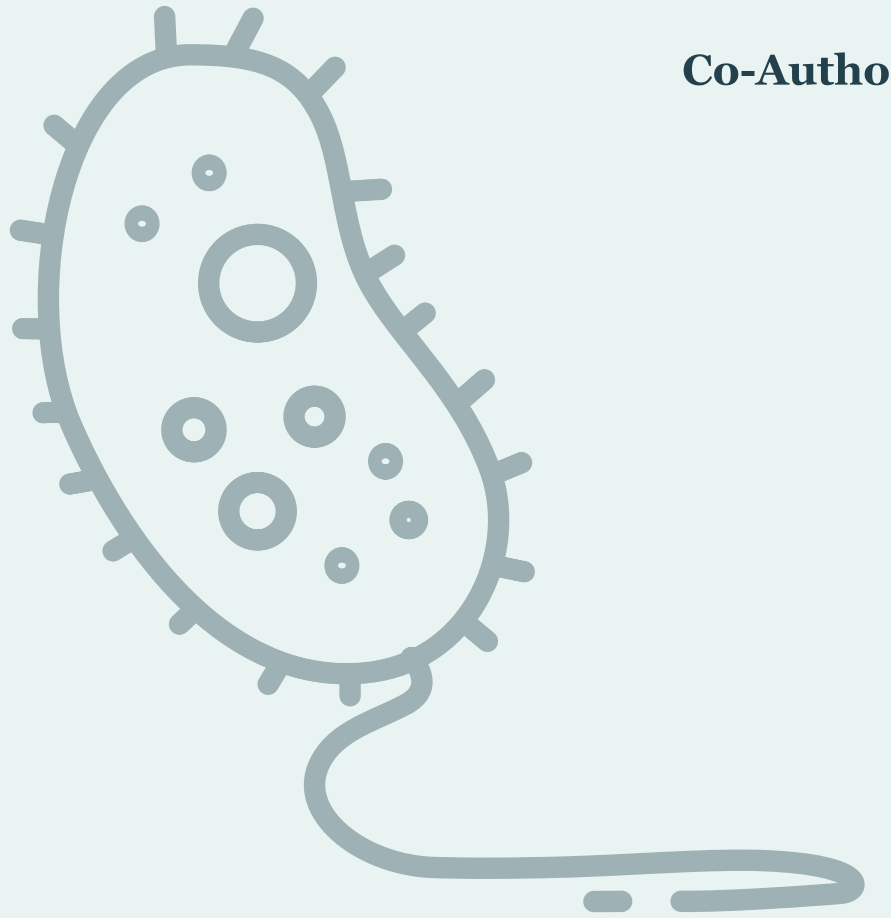
Procedure/Method: Metagenomic and metatranscriptomic analyses were performed to detect high-risk ARGs and quantify their expression activities throughout secondary WWTPs, and compared their fate with that in a local tertiary WWTP. Antibiotic resistance risk scores were calculated by metaCompare.

Findings/Results: Our analysis across influent, denitrifying bioreactor, nitrifying bioreactor, and effluent, identified 28 ARG subtypes, with 10 (35.7%) being high-risk (resistant to aminoglycosides, beta_lactams, chloramphenicol, multi-drugs). Tertiary wastewater treatment exhibited greater efficacy in reducing high-risk ARGs’ gene copies per cell number (GPC) than secondary treatment. Transcriptomic outcomes reveal that despite wastewater treatment diminishing high-risk ARGs’ abundance, the relative gene expression fold change for these in the effluent compared to the influent elevated significantly, averaging 60.35%. This infers that removing high-risk ARGs may be more challenging than low-risk ones., Notwithstanding the risk in effluent being below the environmental baseline, the dispersal of active antibiotic resistance genes and their bacterial hosts in the environment potentially threatens health.

Implications/Applications: This study highlights wastewater treatment plants’ critical role in curbing the environmental dissemination of high-risk ARGs. It provides a detailed overview of high-risk ARGs’ fate throughout treatment processes, crucial for establishing potent strategies combating antibiotic resistance within a “One Health” approach. Our findings can drive wastewater treatment protocol enhancements to limit high-risk ARGs’ impact on human and animal health, while informing source wastewater management.

Author: Guoqing Zhang, Westlake University, China

Co-Authors: Zhang, Zhiguo; Ju, Feng



Session B

Abstract 146

Poster Board number: 117

Flash Poster Presentation: S19 - Thursday, May 30 - 13:45-15:30

Microbial risks triggered through oral administration of antibiotics in fish aquaculture far persist the legally regulated antibiotic withdrawal time

Background: The production intensity in industrial aquaculture systems is regularly challenged by the threat of disease outbreaks. Consequently, antibiotics are widely introduced to ensure meeting the production demand. However, the attention about microbial risk of antibiotic therapy was limited, especially after the withdrawal time.

Procedure: To simulate the effects of standard-length and prolonged antibiotic therapy on Cyprinus carpio, a fish commonly farmed worldwide, florfenicol treatments of different length were applied in tank-based production systems. Time-series metagenomic analyses were conducted to decipher the succession patterns of the resistome, mobilome, and microbiome in the Cyprinus carpio gut.

Results: Remarkable shifts in the resistome were caused by the oral administration of florfenicol and significantly higher antibiotic resistance gene (ARG) abundances remained after the regulated withdrawal time under both standard and prolonged therapy when compared to the control group. Diverse pathways of ARG dissemination facilitated through diverse mobile genetic elements (MGEs) were identified in the treatment groups. A conjugative plasmid and complete integrons with diverse ARGs were regularly detected during the standard therapy. During prolonged therapy, a composite transposon containing floR was identified. Even after the regulated withdrawal time, the period after antibiotic therapy that fish can be used for consumption, identified plasmids in fish gut microbiota carried more diverse ARGs compared to the control. Each treated groups was throughout stably colonized with ARG-carrying Enterobacter which is human opportunistic pathogen. For the prolonged therapy group opportunistic pathogens including Escherichia and Salmonella carrying multiple ARGs were also more highly abundant compared to the controls.

Implications: These results indicate that microbial risk factors are trigged by oral administration of florfenicol and persist longer than the suggested withdrawal times, suggesting that when determining safe withdrawal times in aquaculture, increased considerations should be given to microbial risk factors and especially antibiotic resistance to limit the proliferation of ARGs.

Author: Bing Li, Tsinghua University, China

Back to the Program

Session B

Abstract 218

Poster Board number: 118

Flash Poster Presentation: S16 - Thursday, May 30 - 8:30-10:15

Seeds act as vectors for antibiotic resistance gene dissemination in a soil-plant continuum

Background and Aim: Though the evidence of antibiotic resistance spread via plant microbiome is mounting, studies regarding antibiotic resistome in plant seeds, a reproductive organ and important food resource, is still in its infancy.

Method: This study utilized Illumina sequencing of the 16S rRNA gene and High-throughput quantitative PCR (HT-qPCR) to examine the impacts of long-term organic fertilization on seed bacterial endophytes and resistomes in a field experiment, and their inter-generational transfer was also studied in a microcosm pot experiment.

Findings: A total of 99 antibiotic resistance genes (ARGs) and 26 mobile genetic elements (MGEs) were detected in the seed endosphere. The amount of organic fertilizer applied was positively correlated with the number and relative abundance of seed-associated ARGs and MGEs. Moreover, the transmission of ARGs from the rhizosphere to the seed is mainly mediated by shared bacteria and MGEs. Notably, the rhizosphere of progeny seedlings derived from seeds harboring abundant ARGs was found to have a higher relative abundance of ARGs. Using structural equation models, we further revealed that seed resistome and MGEs were key factors affecting the ARGs in progeny rhizosphere, implying the seed was a potential resistome reservoir for rhizosphere soil.

Implications: Our findings emphasize the overlooked role of seed endophytes in the dissemination of the resistome in the soil-plant continuum. Considering that seeds are not only the reproductive organs of plants but also a staple food for humans and some animals, there is an urgent need to give increased consideration to plant seeds as vectors of ARGs within the "One-Health" framework.

Author: Xin-Yuan Li, Zhejiang University, China

Co-Authors: Wu, Wei-Feng; Lin, Xian-Yong; Zhu, Yong-Guan

Session B

Abstract 127

Poster Board number: 120

Antimicrobial Resistance Profiling of Enteric Bacterial Pathogens in Sheep and Goats at a Livestock Show

Background: Livestock exhibitions may serve as unintentional avenues for inter-farm transmission of antibiotic-resistant pathogens. The aim of this study was to profile resistance and virulence markers in sheep and goat feces from a live animal show corresponding to Escherichia coli, Campylobacter spp., Salmonella spp. and Mycobacterium avium paratuberculosis (MAP).

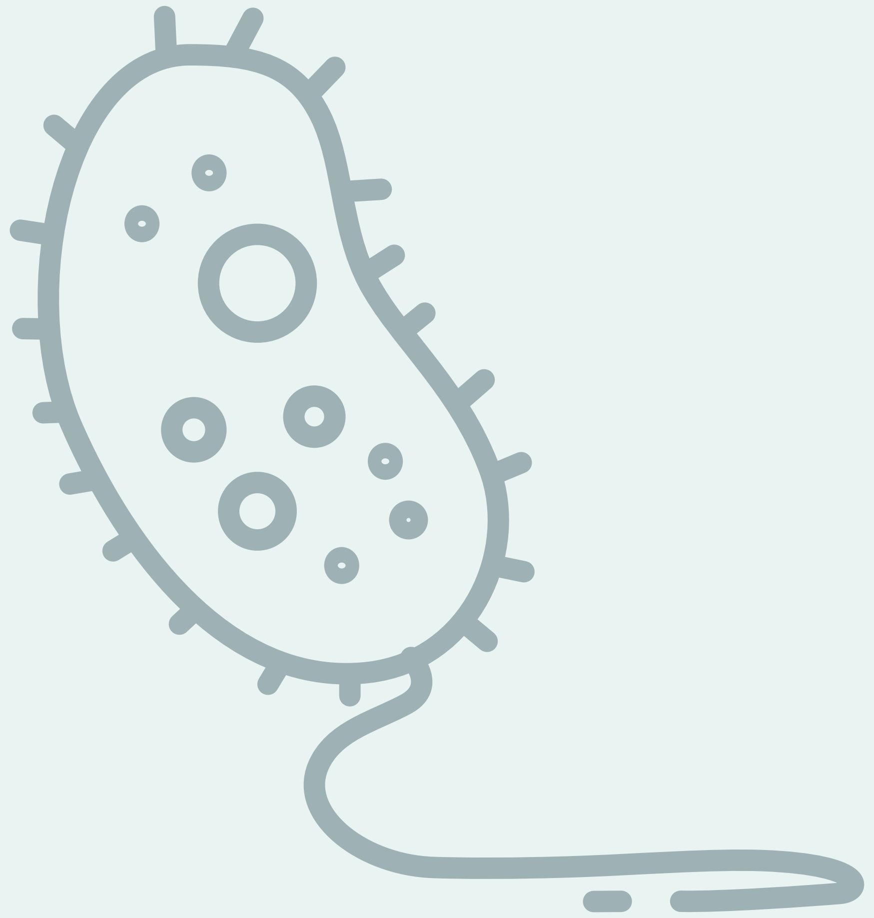
Procedure/Method: Fecal samples were collected from 44 animals representing 16 distinct farms at an annual sheep and goat show and transported on ice to the laboratory. DNA was extracted from fecal samples and from tryptic soy broth overnight enrichments. PCR was applied to detect specific pathogen, antibiotic resistance, and virulence markers, including Campylobacter, Salmonella, E.coli and MAP.

Findings/Results:Although all animals appeared healthy at the time of sampling, concerning bacterial strains were detected in their feces. Shiga toxin-producing E. coli (STEC) exhibited an animal and farm prevalence of 20% each, of these, five animals (11%) harbored the shiga toxin (stx) 1 gene, nine animals (20%) harbored the stx2 gene, and five animals (11%) harbored both stx1 and stx2 genes. The intimin gene was detected in eight animals (18%) from six different farms (38%). In six of these animals, the gene was detected alongside shiga toxin genes in the same animal. Campylobacter was detected in 45% of the animals from thirteen of the sixteen farms (81%), while 11% of animals were positive for MAP from five different farms (31%). No Salmonella positive animals were detected. Resistance profiling is underway.

Implications/Applications: These findings highlight carriage of economically/medically impactful pathogens among small ruminants in Agro-Systems and livestock shows as a potential forum for transmission. While it is often assumed that antimicrobial use and AMR in small ruminants is minimal, this study will help verify this assumption. Addressing this challenge will require a One Health approach to mitigate potential risks to livestock and public health.

Author: Ivan Odur, Virginia Polytechnic Institute and State University, United States

Co-Authors: Pruden, Amy; Pithua, Patrick; Ndegwa, Eunice; Kim, Chyer; O'Brien, Dahlia



Back to the Program

Session B

Abstract 327

Poster Board number: 122

Flash Poster Presentation: S10 - Tuesday, May 28 - 13:45-15:30

What risk does crop irrigation with reclaimed municipal wastewater represent with respect to the transmission of antimicrobial resistance to humans?

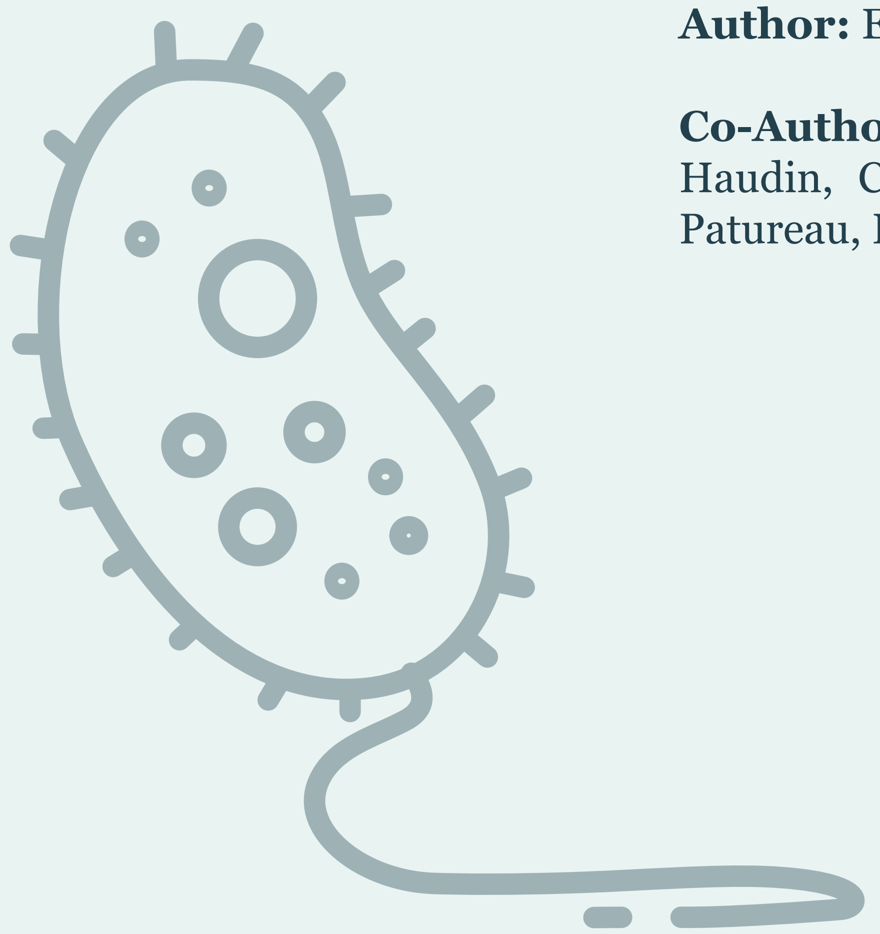
Background and Aim: Climate change associated water stress in the dry southern regions of Europe, Australia and the US South West is threatening food security. In this context, irrigation of crop ground with effluent from municipal wastewater treatment plants is a practice that is become more widely adopted. Sewage effluents can contain pharmaceuticals, other micropollutants, and enteric bacteria including those that carry antibiotic resistance genes. The European Commission has recently set standards for the quality of effluents to be used in crop production with the abundance of viable E. coli as the only microbiological endpoint of concern. https://environment.ec.europa.eu/publications/minimum-requirements-water-reuse-guidelines_en. There is currently no provision for including risk of AMR transmission in the standards. Therefore, the key aim of the work considered here is to provide policy makers with evidence concerning what risk this practice might represent from the perspective of AMR transmission, and options for managing the risk of transmission.

Objectives: In 2023 the medical research council of France (INSERM) funded a research team (the MEHTA project; managing environmental hotspots and transmission of AMR) to evaluate the sewage treatment-to-farm-to-gut risk of AMR transmission from crop production systems that are irrigated with sewage effluent. Outcomes of the project include 1. Impact of tertiary treatment methods on water microbial and chemical quality; 2. Persistence of microbial and chemical contaminants in crop production systems; 3. Predicted no effect concentrations (PNECs) of antibiotics entrained into soils; 4. Microbiological and chemical quality of irrigated crops. 5. Impacts of consumption of irrigated crops on the resistome and mobilome of the human gut microbiome.

Implications: Results from the MEHTA project will help inform policy and practice with respect to water reuse from the perspective of AMR transmission risk.

Author: Ed Topp, INRAE, France

Co-Authors: Alvarez-Fraga, Laura; Benoit, Pierre; Deschamps, Marjolaine; Hartmann, Alain; Haudin, Claire-Sophie; Hocquet, Didier; Le Maréchal, Caroline; Martin-Laurent, Fabrice; Patureau, Dominique; Martak, Daniel



Session 13

Abstract 150

Poster Board number: 123

Unravelling the role of elevated soil temperature and the application of biocides on the spread of antibiotic resistance in organically amended soils

Background and Aim: Antibiotic resistance and climate change are prominent threats to human and environmental health, often studied in isolation. Biocides used in agriculture can affect soil microbial communities and contribute to the co-selection of antibiotic resistance. The objective of this study was to examine the combined effects of elevated soil temperature and the application of fungicides and herbicides on the emergence and spread of antibiotic resistance in organically amended soils.

Procedure/Method: In a field experiment, potatoes were grown in a split-plot design with six replicates per treatment. To simulate climate change, soil temperature was elevated by 5°C compared to normal conditions. During crop development, half of the plots received seven fungicide and herbicide treatments, while the others served as controls. All plots were amended with aged cow manure. The ResCap targeted sequence capture platform was used to assess genotypic changes in soil and plant resistomes and mobilomes, covering up to 78,600 non-redundant genes, including antimicrobial resistance genes and mobile genetic elements. Phenotypic changes were evaluated through the determination of minimum inhibitory concentrations using the microdilution method. DNA metabarcoding was used to analyse the composition of soil microbiomes. Soil physicochemical and microbial parameters were determined to assess the effect of the applied treatments on soil functioning.

Findings/Results: Our results show statistically significant changes in the soil resistome due to the interactive effects of elevated soil temperature and biocide application.

Implications/Applications: This integrated approach has provided insights for a better understanding of antibiotic resistance emergence and dissemination in agricultural settings, considering the influence of both climate change and agricultural practices. The knowledge thus obtained can help design better strategies for the control of antibiotic resistance spread and, concomitantly, enhance environmental and human health.

Author: Fernando Ruiz Torrubia, Neiker, Spain

Co-Authors: Anza Hortala, Mikel; Anitua Martínez, Aitor; Olavarrieta Scappini, Leticia; Fernández, Val; Coque, Teresa; Garbisu Crespo, Carlos; Epelde Sierra, Lur

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 25

Poster Board number: 124

Occurrence and Spread of PPCPs and Antibiotic Resistance in Urban Waters of Sri Lanka

Background and Aim: Emerging pollutants, including Pharmaceuticals and Personal Care Products (PPCPs), in water resources are now recognized as a worldwide water quality concern. To effectively manage PPCPs, including Antibiotics, we must understand their sources, fate, and how they move in urban water systems. The emergence of Antibiotic-Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARGs) in natural water bodies is consistently highlighted as a significant public health issue. However, research on PPCPs in urban waters and their utilization for monitoring wastewater pollution in developing nations such as Sri Lanka has been limited. We have undertaken various studies to determine the prevalence of PPCPs, ARB, and ARG in urban waters in Sri Lanka.

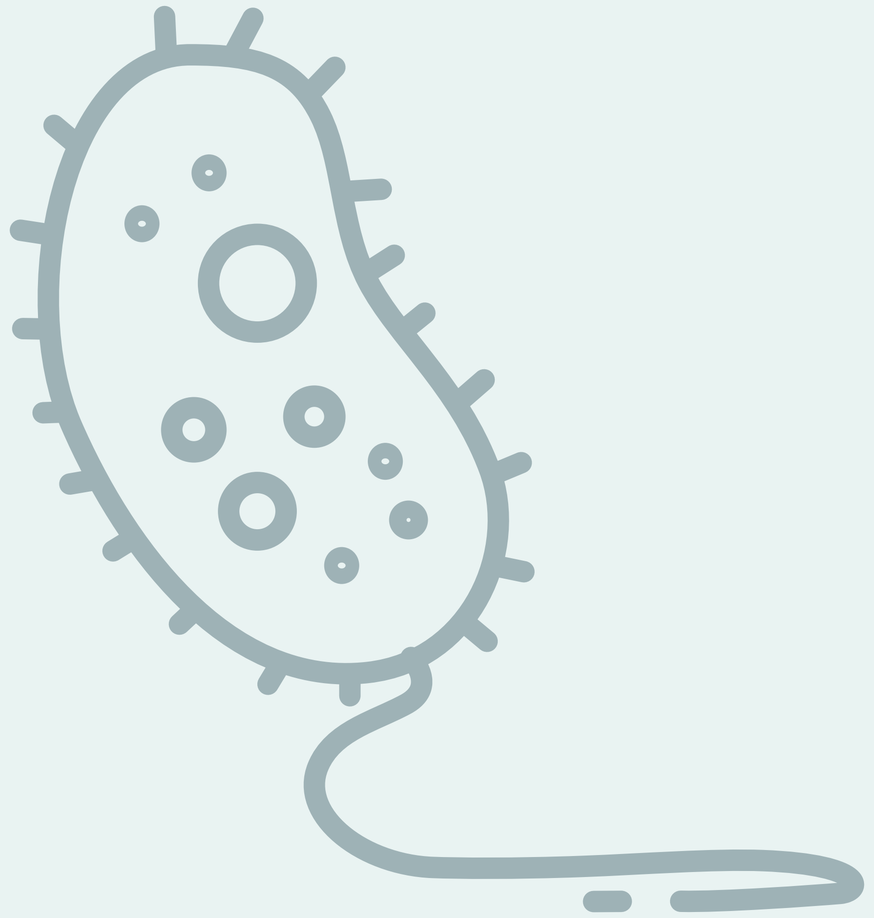
Procedure/Method: Samples from urban rivers, canals, and influents and effluents of wastewater treatment plants (WWTP) in Colombo and Galle cities in Sri Lanka were analyzed for PPCPs and ARBs. The antibiotic susceptibility test was carried out using the KB disk diffusion method.

Findings/Results: We observed the presence of approximately 20 different PPCPs, with concentrations reaching up to 124 µg/L, in urban waters across Sri Lanka. Almost all sampling points in Sri Lanka contained E. coli strains that resisted more than one antibiotic. Notably, antibiotic resistance in urban waters in Sri Lanka falls between 20% and 100%, significantly higher than in other Asian countries. Effluents of WWTPs in Sri Lanka showed more antibiotic resistance and a consistent increase in antibiotic resistance after the treatment. Further, we observed that compared to the effluents from municipal WWTPs, the hospital WWTP had a higher proportion of multidrug resistance, which can be due to the higher concentration of pharmaceutical products in the hospital wastewater.

Implications/Applications: This is a matter of concern, as it signals the emergence of multidrug-resistant bacterial strains as a substantial threat to public health within the community.

Author: GG Tushara Chaminda, University of Ruhuna, Sri Lanka

Co-Authors: Kumar, Manish; Honda, Ryo; Otaki, Yurina; Otaki, Masahiro



Session B

Abstract 332

Poster Board number: 125

Fate of mobilized colistin resistance genes with increasing concentration of last resort antibiotic in anaerobic membrane bioreactors

Colistin, also known as polymyxin E, is an antimicrobial agent effective against various Gram-negative bacteria. Recently, the broad dissemination of colistin resistance has drawn attention due to its importance as a last resort for treating carbapenem-resistant Enterobacteriaceae infections and the potential for horizontal transfer. Mobilized colistin resistance (mcr) genes were first discovered in 2015 and now 10 genes (mcr 1-10) have been identified worldwide. The present work aims to examine the response of mcr genes to changing colistin selective pressure and associated changes to the microbial community in anaerobic membrane bioreactors (AnMBRs) treating low-strength wastewater. Colistin was added at incremental concentrations of 10, 50, and 100 ug/L for 10 days each to a bench-scale AnMBR. System performance was consistent including chemical oxygen demand (COD) removal averaging 91.5 ± 1.8 %. Mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) concentrations, and pH were relatively constant at 8.7 ± 1.5 g/L, 6.6 ± 1.42 g/L, and 6.5-7.3, respectively. Average biogas production was 550 ± 12 mL/d with an average methane content of 78.0 ± 0.9%. A novel duplex-droplet digital PCR assay was developed for mcr 1-10 quantification. Results revealed positive mcr-3, mcr-4, mcr-5, mcr-6, mcr-9, and mcr-10 detection in biomass and biofilm samples. Sequencing of 16S rRNA and 16S rDNA genes indicated that sludge (biomass and biofilm) and effluent had distinctly different microbial community structure and activity profiles. In effluent samples, Sulfurovum and Pseudarcobacter showed increasing activity with the addition of colistin. Anaerolineaceae, Clostridiales, Firmicutes, and Flavobacterium were dominant while Sytrophaceae, Prolixibacteraceae, and Rectinema significantly decreased in the presence of colistin as well as in attached growth.

Author: Harmita Golwala, University of Southern California, United States

Co-Authors: Saldana, Michael; Smith, Adam

Back to the Program

Session B

Abstract 182

Poster Board number: 131

Surveillance on dynamic patterns of antibiotic resistant bacteria and multi-drug resistance in Sagami River, Japan

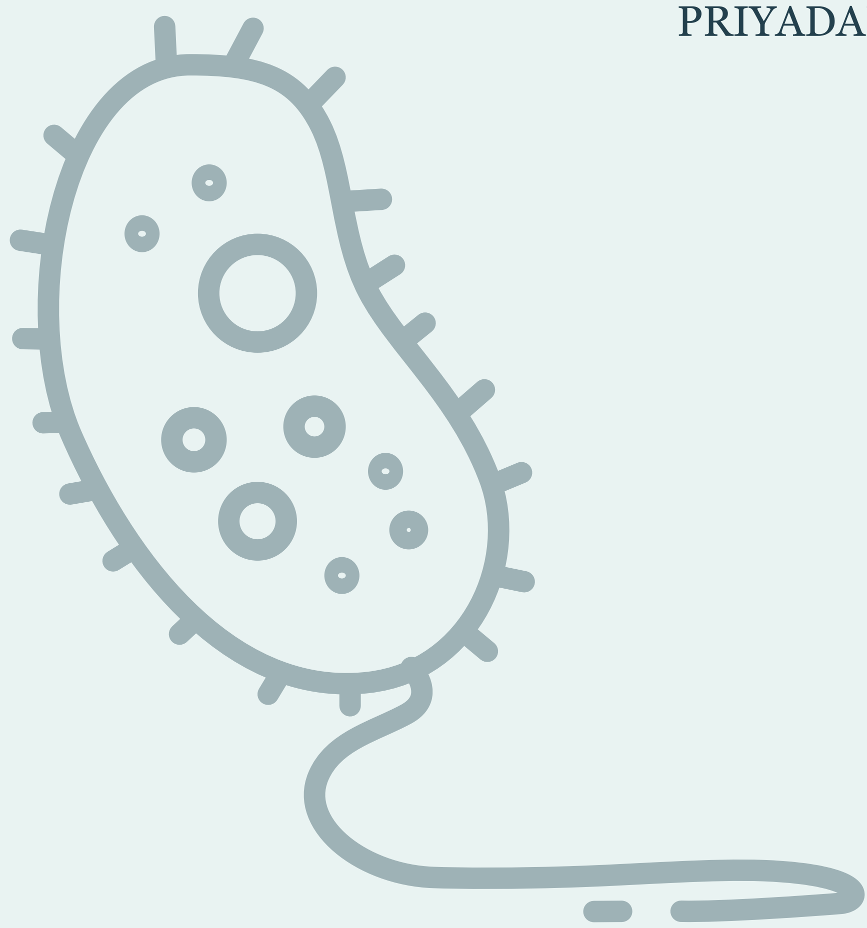
Background: Antibiotic Resistance (AR) poses a significant risk to human health. Aquatic environments have been identified as a major route of AR dissemination. Understanding the pattern of the variation of AR in natural waterbodies is vital to identify the sources or origins and its dissemination in ecosystems. This study focused on identifying the spatial and temporal trends of AR in Sagami River as an initiative to detect the sources of origins of AR.

Method: 1728 heterotrophic bacteria were isolated from the water collected at six locations of the Sagami River, covering upstream, midstream, and downstream. in autumn of 2020–2022. Isolates were identified by 16S rRNA sequencing, and antibiotic resistance profile of each isolate for 17 antibiotics belonging to Penicillin, Cephalosporins, Carbapenems, Glycopeptides, Aminoglycosides, Tetracyclines, Chloramphenicol, Macrolides, and Quinolones groups were checked by the disk diffusion method based on CLSI guideline.

Results and Implications: Acinetobacter sp., Aeromonas sp., and Pseudomonas sp. were the most dominant species in 2020, 2021, and 2022, respectively, where their resistance patterns varied from upstream to downstream. The highest resistance was observed for Cephalosporins, followed by Penicillin, Macrolides, and Quinolones (the most clinically prescribed antibiotics in Japan), and the least resistance was observed for Carbapenems, followed by Glycopeptides and Tetracyclines. The midstream showed the highest resistance ratio in general, while the downstream showed the lowest. An increasing trend in resistance ratio for all the tested antibiotics was observed from 2020 to 2022. Clinically important multidrug-resistant (MDR) bacteria were observed throughout the Sagami River where an increase trend of MDR was observed in both clinically important MDR bacteria and the dominant Antibiotic Resistant Bacteria (ARB) populations. Further investigation is needed to confirm whether these observed trends reflect the prevailing situation, and to identify the possible sources and mechanisms of generation and transmission of MDR-ARB in aquatic environments.

Author: Kazunari Sei, Kitasato University, Japan

Co-Authors: OGINO, Mizuki; Amarasiri, Mohan; FURUKAWA, Takashi; KAMEI, Tatsuru; PRIYADARSHANI, Chandrika



Session B

Abstract 261

Poster Board number: 132

Determination of resistance cut-off concentrations for the biocides didecyldimethylammonium chloride (DDAC) and methylisothiazolinone (MIT)

Background: Biocides are important to prevent microbial contamination and infectious disease. However, exposure to biocides has been linked to the co-selection of antimicrobial resistance. So far, there are no standardized methods or criteria to investigate biocide resistance. Here, we aimed to determine resistance cut-off concentrations for two commonly used and chemically unrelated biocides: didecyldimethylammonium chloride (DDAC) and methylisothiazolinone (MIT).

Method: We determined resistance cut-off concentrations for DDAC and MIT in E. coli using native wastewater communities and pure isolates. Cut-offs were defined as biocide concentration at which the majority of naturally occurring E. coli strains cannot grow anymore based on (i) the minimal inhibitory concentration (MIC) distribution, and (ii) by plating wastewater communities onto selective agar plates loaded with increasing biocide concentrations.

Results: MIC testing of E. coli isolates by broth microdilution revealed a resistance cut-off concentration of ≥ 6 µg/ml DDAC and ≥ 12.5 µg/ml MIT corresponding to the highest MIC in the tested population. Based on community plating, a cut-off concentration of 200 µg/ml DDAC and 6.3 µg/ml MIT was determined at which a growth reduction of 99.9 % and 99.5 % was observed. For DDAC, the correlation between the two cut-off concentrations in broth and agar could be verified by agar dilution method. Accordingly, E. coli strains isolated from selective DDAC plates showed a MIC distribution shifted to higher concentrations with single isolates having MIC values 8-fold higher than the resistance cut-off concentration. In contrast, selection on MIT agar plates could not be related to changes in the MIC.

Implications: Our results show the challenges in determining resistance cut-off concentrations for biocides and the importance of control experiments. Determining a selective concentration for DDAC which is reflected in changes in the MIC level enables us to investigate co-selection between biocide and antibiotic resistance in natural microbial communities.

Author: Anne-Christine Schulz, Federal Institute for Material Research and Testing, Germany

Co-Authors: Staravoitava, Anastasia; Schreiber, Frank

Session B
Abstract 137

Poster Board number: 134

Flash Poster Presentation: S17 - Thursday, May 30 - 11:00-12:30

The effect of propidium monoazide (PMA) pre-treatment on qPCR-based pathogen survival monitoring of ozone-treated wastewater effluent

Background and Aim: Quantifying viable bacteria or pathogens in environmental samples is an important challenge in public health studies due to viable but non-culturable (VBNC) bacteria in culture-based methods and overestimation in conventional DNA-based techniques. Propidium monoazide quantitative polymerase chain reaction (PMA-qPCR) is a promising method to quantify only viable bacteria, including VBNC pathogens, in environmental samples.

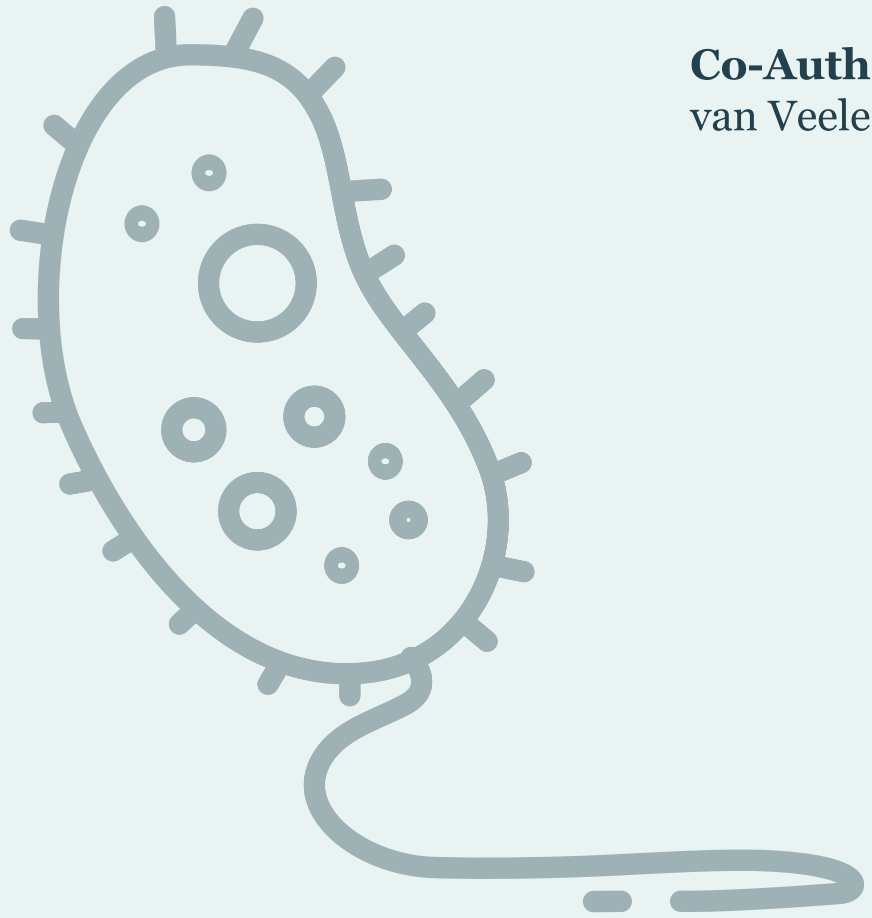
Procedure/method: A PMA method has been adapted and used to validate (i) the application of PMA-qPCR for single Gram-positive and Gram-negative bacteria in a range of live-dead proportions (100, 10, 1, 0.1 and 0% living cells) and (ii) for species surviving in ozone treated wastewater effluent spiked with either E. coli or P. aeruginosa strains. Membrane filtered samples were subjected to culturing and qPCR, as well as PMA-qPCR for determination of VBNC bacteria.

Findings/Results: (i) For both Gram-positive and Gram-negative bacteria, with conventional qPCR, the proportions of living and dead cells did not influence the observed concentration. With PMA-qPCR, the observed concentration is proportional to the proportion of live bacteria, indicating the efficacy of PMA application. (ii) In ozonated wastewater effluent spiked with E. coli, a reduction of 4.7 log, 2.6 log and 1.8 log was measured by culturing, PMA-qPCR and conventional qPCR, respectively. Spiked P. aeruginosa was reduced by 1.6 log, 0.8 log and 0.8 log, respectively, supporting better ozone survival. Applying PMA was less efficient for samples containing >99% dead cells and for samples with a low starting total cell concentration (10^5).

Implications/Applications: PMA-qPCR enhances molecular detection of viable pathogens, including VBNC bacteria, in ozone treated effluent and has the potential to be applied to other environmental samples containing dead cells. Further optimization of the PMA method is required for the application on environmental samples with low viable cell concentrations.

Author: Elfy Ly, Wetsus / TU Delft, Netherlands

Co-Authors: van de Pol, Denise; van Loosdrecht, Mark; Hernández Leal, Lucía; Schmitt, Heike; van Veelen, Pieter



Session B
Abstract 371

Poster Board number: 135

Flash Poster Presentation: S6 - Monday, May 27 - 13:45-15:30

Profiling of Resistant Genes in Intermittent Water Distribution Systems and Household Water Storage

Water distribution systems (WDS) provide important health benefits when they provide safe and reliable water; however, they can also harbor pathogens and spread antimicrobial resistance (AMR), posing a threat to public health globally. This concern is heightened for the more than one billion people worldwide who only have access to a WDS that operates intermittently. Intermittent water supply systems (IWS) are piped water supplies that deliver water to consumers irregularly, with hours or days of loss of pressure/service interruption. In IWS, contaminated water can mix with drinking water in pipes through leaks and cracks at no/low pressure. This could spread waterborne pathogens and present opportunities for horizontal gene transfer and co-selection that contribute to AMR between organisms. Furthermore, frequent water service interruption creates conditions for adaptative strategies for water scarcity, such as household water storage. The influence of AMR on health and the understudied practice of IWS demands an extensive investigation. This research aims to understand the role of frequent interruptions and stored water on the prevalence of AMR in WDS and households.

Methods: We used a dead-end hollow-fiber ultrafilter to filter 30-100L of water from Source to Household (source water/treated water/tap water/stored water). Then, we extracted their DNA and sent it to the UMass Genomic Core Facility for shotgun sequencing. Simultaneously, we conducted 200 household surveys to assess patterns of antibiotic use, providing a holistic understanding of AMR dynamics in Machakos, Kenya.

Results: Preliminary analysis of our survey indicates that 64% and 61% of households use azithromycin and amoxicillin/clavulanic acid, respectively. Sequencing run results are anticipated in the spring of 2024.

Implications: By elucidating AMR dynamics in IWS systems and Household Storage, this work will illuminate pathways with crucial public health implications, guiding interventions to safeguard communities from the risks associated with AMR in drinking water systems.

Author: Gabriel Mesole, University of Massachusetts - Amherst, United States

Co-Authors: Chavarria, Karina; Weston, Sally; Kumpel, Emily

Back to the Program

Session B

Abstract 151

Poster Board number: 136

Flash Poster Presentation: S17 - Thursday, May 20 - 11:00-12:30

Assessing the impact of anthropogenic pollution on microbial diversity and AMR in recreational waters using metagenomic sequencing

Background and Aim: Understanding the role of the environment in the persistence and transmission of antimicrobial resistance (AMR) is essential in developing targeted mitigation strategies. Recreational waters may represent a reservoir for AMR transmission to humans. The aim was to perform shotgun metagenomic sequencing to assess the impact of anthropogenic contamination on the receiving environment.

Procedure/Method: Twelve samples of water from a coastal location on the West coast of Ireland receiving untreated wastewater were collected on 4 dates between March and April 2021. Water was collected from 3 points 1) wastewater discharge point (DP) 2) seawater to the west of the DP, and 4) an adjacent freshwater stream. Samples were filtered and DNA extracted for deep shotgun metagenomic sequencing. Bioinformatic analysis was performed to assess microbial diversity, composition, function, and AMR profiles.

Findings/Results: Across all sampling points, up to 1,200 bacterial genera and 6,000 bacterial species were identified. While freshwater and seawater microbiomes were dominated by bacterial species associated with soil, plant roots and algae, the DP was dominated by human enteric bacteria including opportunistic pathogens. Our results highlighted the DP as a source of introduction of pathogens and antimicrobial resistant organisms (AROs) into the environment as all ESKAPE pathogens i.e. E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, and Enterobacter were detected in high abundance both at the DP and in the adjacent communal seawater, as well as AMR genes of clinical significance i.e. carbapenemase-encoding genes (blaNDM-1) at the DP.

Conclusions: The results provide valuable insights into the impact of anthropogenic activity on recreational waters and support the use of metagenomic sequencing in the surveillance of such environments. It is evident that the release of untreated wastewater contaminated with pathogens and AROs both negatively impact microbial communities in these waters and pose a risk to public health.

Author: Dearbháile Morris, University of Galway, Ireland

Co-Authors: Cahill, Niamh; Miliotis, Georgios; O’Connor, Louise; Hooban, Brigid

Session B

Abstract 382

Poster Board number: 137

Paper microfluidic device for rapid detection of antimicrobial resistance in water

Background and Aim: The presence of antimicrobial resistance (AMR) in water is one of the global concerns on environmental and human health. AMR occurs when microorganisms that cause infection survive exposure to drugs that would normally kill them or stop their growth. In particular, the overuse of antimicrobials has increased the AMR spreading and transmission in the environment. This project aims to develop a multiplexed recombinase polymerase amplification (mRPA) - clustered regularly interspaced short palindromic repeats (CRISPR)/Cas assay to obtain ultra-sensitive and specific detection of tetracycline and emerging tigecycline resistance genes, as well as Crassphage, human-specific biomarker, for source tracking.

Procedure/Method: We present a cost-effective, rapid, and easy-to-use approach for the detection of bacteria and ARGs in drinking water by combining microfluidic paper-based analytical devices (µPADs) and RPA coupled with CRISPR)/Cas. RPA exponentially replicates template genes for sensitive detection with efficient multiplexing capability. CRISPR/Cas system then recognises individual specific target sequences with designed guide RNA, which then triggers its trans-cleavage activity with multiple turnovers, further enhancing on the sensitivity of the assay. We firstly detected pathogenic organisms in drinking water with a detection limit of 10 copies DNA per reaction.

Findings/Results: Our device was able to detect MCR-1 gene, CTX-M-2 gene and NDM-1 gene at 10 copies of DNA per reaction within less than 2 hours. Target DNA was amplified by RPA and detected fluorescently using a hand-held UV torch. A mobile phone was used to capture images for both quantitative and qualitative analysis, enabling onsite detection.

Implications/Applications: The paper sensor can be integrated into field application modules to establish new avenues for on-site sample preparation and rapid detection of bacteria and ARGs, which holds great promise for routine monitoring of antimicrobial resistance in drinking water, especially at resource-limiting areas, e.g., low- and middle-income countries to contribute global health challenges.

Author: Zhugen Yang, Cranfield University, United Kingdom

Co-Authors: Pan, Yuwei

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 39

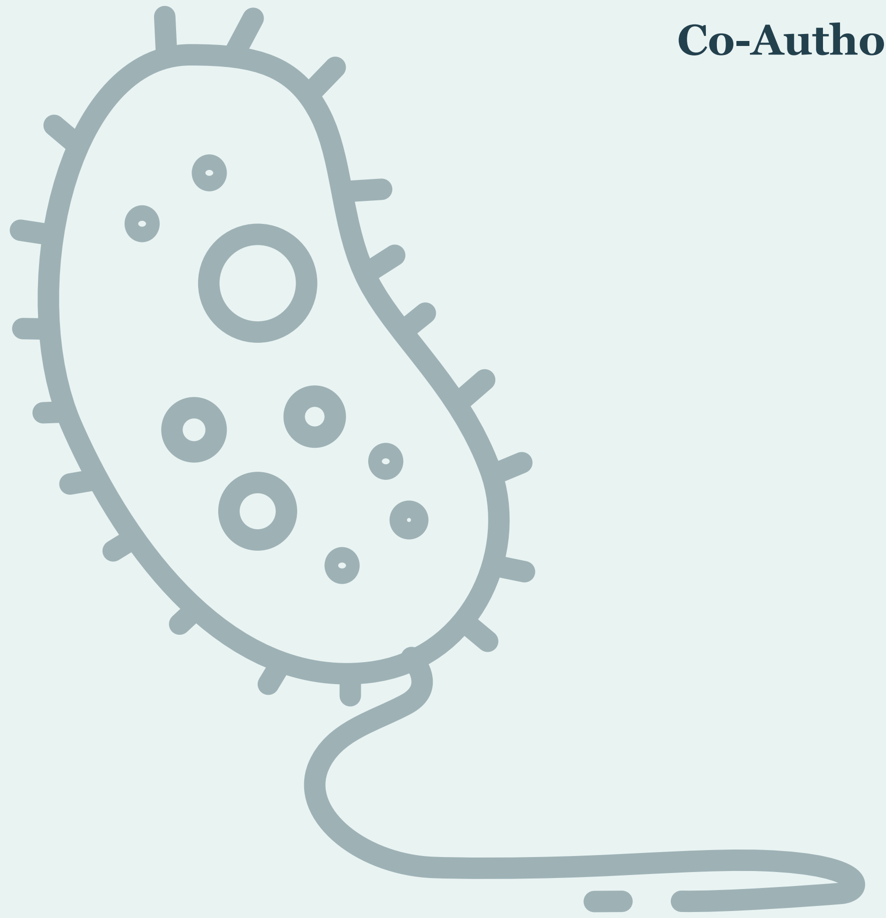
Poster Board number: 141

The fate of high-risk antibiotic resistance genes in secondary and tertiary municipal wastewater treatment plants

"Background and Aim: Wastewater treatment plants (WWTPs) are important sources of antibiotic resistance genes (ARGs) in the environment. High-risk ARGs, which refer to those transferable ones enriched in human-related environments and accessible by pathogenic bacteria, pose a more direct and significant threat to human and animal health. Therefore, understanding the fate and expression of high-risk ARGs during wastewater treatment processes, are the prerequisites for effectively combating antibiotic resistance in the “One Health” framework. Procedure/Method: Metagenomic and metatranscriptomic analyses were performed to detect high-risk ARGs and quantify their expression activities throughout secondary WWTPs, and compared their fate with that in a local tertiary WWTP. Antibiotic resistance risk scores were calculated by metaCompare. Findings/Results: Our analysis across influent, denitrifying bioreactor, nitrifying bioreactor, and effluent, identified 28 ARG subtypes, with 10 (35.7%) being high-risk (resistant to aminoglycosides, beta_lactams, chloramphenicol, multi-drugs). Tertiary wastewater treatment exhibited greater efficacy in reducing high-risk ARGs' gene copies per cell number (GPC) than secondary treatment. Transcriptomic outcomes reveal that despite wastewater treatment diminishing high-risk ARGs' abundance, the relative gene expression fold change for these in the effluent compared to the influent elevated significantly, averaging 60.35%. This infers that removing high-risk ARGs may be more challenging than low-risk ones., Notwithstanding the risk in effluent being below the environmental baseline, the dispersal of active antibiotic resistance genes and their bacterial hosts in the environment potentially threatens health. Implications/Applications: This study highlights wastewater treatment plants' critical role in curbing the environmental dissemination of high-risk ARGs. It provides a detailed overview of high-risk ARGs' fate throughout treatment processes, crucial for establishing potent strategies combating antibiotic resistance within a ""One Health"" approach. Our findings can drive wastewater treatment protocol enhancements to limit high-risk ARGs' impact on human and animal health, while informing source wastewater management."

Author: Guoqing Zhang, Westlake University, China

Co-Authors: Zhang, Zhiguo; Ju, Feng



Session B

Abstract 358

Poster Board number: 143

Understanding Antibiotic Purchasing Practices in Community Pharmacies in Bangladesh: A Potential Driver of Emerging Antimicrobial Resistance.

Antimicrobial resistance poses significant health threats for low-and-middle-income countries and 80% of antibiotics are used in the community, with 20-50% inappropriate use. This research utilized cross-sectional survey (N=385) involving antibiotic customers from sampled urban and rural pharmacies across four sites in Bangladesh, excluding hospital-adjacent areas to mitigate potential biases of formal healthcare medication patterns. Descriptive-analyses were employed to characterize antibiotic purchases, while Poisson-Regression-analysis identified associations with disease, demographic factors, and the likelihood of having a prescription during antibiotic purchases. Approximately 56.6% antibiotics obtained without a prescription from drug-sellers, self-medication, and non-registered healthcare providers, mostly for non-severe health-conditions like fever and upper-respiratory-tract-infections (23.4%), uncomplicated skin-infections (14.1%), gastrointestinal-infections (8.5%), urinary-tract-infections (7.9%) and various other symptoms. Frequently purchased antibiotics included macrolides (22.1%), third-generation (20.8%), and second-generation (16.9%) cephalosporins. Following the WHO-AWeRe classifications, 73.8% of purchased antibiotics fell into the Watch, 25.5% were in the Access category, and notably, 6.23% were broad-spectrum not-recommended antibiotics. The likelihood of having a prescription while purchasing antibiotics is significantly lower (Adj.-PR=0.7, 95% CI:0.54, 0.90) for individuals aged 6-59 compared to those in the disease-susceptible age group (≤ 5 or ≥ 60). Demographic factors such as sex, urban-rural residence, education, household income, and the number of health-symptoms were not statistically significant after adjusting confounders. The prescription likelihood for antibiotics varied across health-symptoms, with conditions like lower-respiratory-tract-infections (Adj.-PR=1.83, 95% CI:1.09, 3.07), Tonsillitis (Adj.-PR=1.85, 95% CI:1.01, 3.39), and infection in the eyes (Adj.-PR=2.05, 95% CI:1.18, 3.56) had a higher likelihood of having prescriptions compared to non-severe conditions. This study underscores the widespread antibiotic sales without prescription in Bangladesh. Despite WHO’s 60% recommendation for Access-group antibiotics, they were underused, while the Watch-group was overused. Targeted interventions should focus on user-centric outcomes for non-severe health-conditions in informal healthcare settings. A comprehensive strategy involves tailored communication, education, and awareness programs considering diverse socioeconomic contexts.

Author: Abdullah Al Masud, University of New South Wales, Australia, Australia

Co-Authors: Seale, Holly; Walpola, Ramesh; Sarker, Malabika; Kabir, Alamgir; Asaduzzaman, Muhammad; Mostafa, Ayesha Tasnim; Islam, Md. Saiful; Barua, Mrittika; Akhtar, Zubair

Back to the Program

Session B

Abstract 366

Poster Board number: 144

Confronting AMR: A Comprehensive Approach through Public Awareness and Scientific Engagement

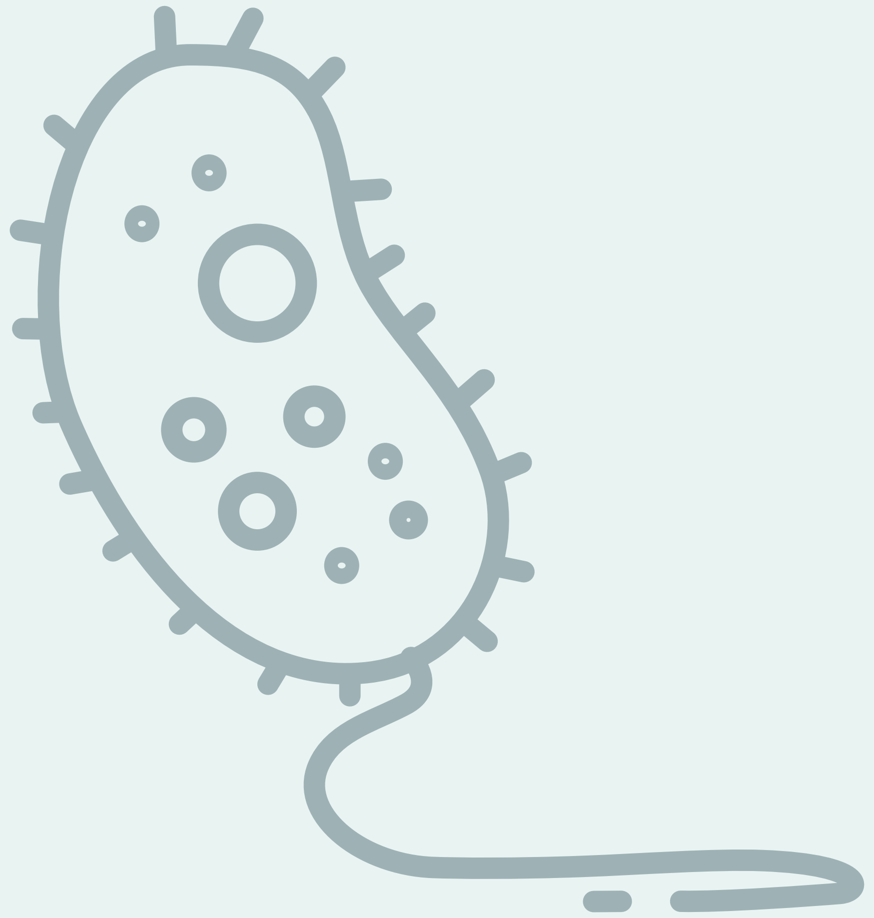
Background/Aim: The antimicrobial resistance (AMR) crisis requires a comprehensive approach extending beyond the mere scientific development of novel antimicrobials. The McGill AMR Centre Student Outreach Team (SOT) addresses a fundamental issue: the lack of public awareness on this critical topic. The SOT aims to increase awareness and cultivate a deeper understanding of the consequences of AMR. The SOT actively engages, educates, and inspires students and the public, encouraging their involvement in the fight against AMR. In our pursuit to confront this global crisis, our aim is clear: enlighten the public and foster awareness about AMR. We aspire to proactively address and mitigate the burden of AMR through social involvement. We believe that every innovation, idea, and contribution is indispensable. By harnessing the power of unity and collective action, we can make significant strides in combating AMR and safeguarding the health of our planet.

Procedure/Method: Student and public outreach events are disseminated through newsletters, social media posts, and student engagement activities. We share informative, relatable, and easily digestible subject matter tailored to resonate with diverse audiences. Our success in building a substantial following is attributed to milestone events such as seminars, symposiums, competitions, and student opportunities.

Findings: Strategically designed campaigns have captured considerable attention and expanded the impact within the community. Emphasizing scientific outreach and awareness has had remarkable success. The disseminated information has experienced a 35% surge in social media growth. Moreover, the target for student participation in events and competitions was surpassed by 70%, reaching students from diverse departments across the university.

Implications: Addressing the AMR crisis requires the integration of both scientific and social dimensions. The student outreach team bridges the gap between the public and scientific communities. Our goal is to actively engage in the battle against AMR through numerous educational, social and outreach initiatives.

Author: Salam, Humza, Arianna Giurleo, McGill University AMR Centre, Canada



Session B

Abstract 409

Poster Board number: 145

Wastewater genomic surveillance of sexually transmitted infections and their antibiotic resistance in Los Angeles County

Background and Aim: Wastewater genomic surveillance allows for a comprehensive spatiotemporal view of a population's infection rates and antibiotic resistance patterns. Chlamydia, gonorrhea, and syphilis are introduced into wastewater systems by bacterial shedding from infected individuals. Typically, the three sexually transmitted infections (STIs) are curable with antibiotics; however, misuse and over-prescription of antibiotics are causing antibiotic-resistant genes (ARGs) to emerge. With the presence of multiple drug-resistant STIs becoming more prevalent, understanding which antibiotics to prescribe in a community is vital.

Procedure/Method: 24-hour composite wastewater samples are taken from the Hyperion Water Reclamation Plant and the University of Southern California. DNA is extracted using HA Filtration and quantified using ddPCR to confirm the presence of STIs and track their prevalence. Further, we perform long-read whole-genome sequencing using Oxford Nanopore Technologies PromothION 2. We are able to identify the ARGs present in the target STIs' chromosomes and plasmids using our bioinformatics pipeline. This pipeline was used to analyze the SRA database for the three STIs to establish the most prevalent ARGs.

Findings/Results: By identifying the ARGs present, we are able to determine which antibiotic drug classes are ineffective for treating each of the three STIs in our study populations. Comparative statistical analysis between building-scale and county-scale and their respective clinical data allows us to determine the scale of surveillance that assists prevention protocols most effectively.

Implications/Applications: In conjunction with public health officials, wastewater genomic surveillance of chlamydia, gonorrhea, and syphilis can help with prevention and treatment by detecting spikes within the population and identifying which antibiotics should be excluded from treatments. Wastewater genomic surveillance allows for passive surveillance that can capture antibiotic resistance before clinical testing.

Author: Michael Saldana, University of Southern California, United States

Co-Authors: Smith, Adam

Thursday, May 30 - 16:00-18:00

Back to the Program

Session B

Abstract 116

Poster Board number: 146

Flash Poster Presentation: S7 - Tuesday, May 28 - 8:30-10:21

Opinions of stakeholders on the government, regulator and water industry response to the environmental challenges on the UK AMR National Action Plan

Background and Aim: The United Kingdom (UK) currently has a 5-year National Action Plan (NAP) that runs from 2019 to 2024. This study aimed to assess how well the UK had achieved the environmental goals presented in this NAP.

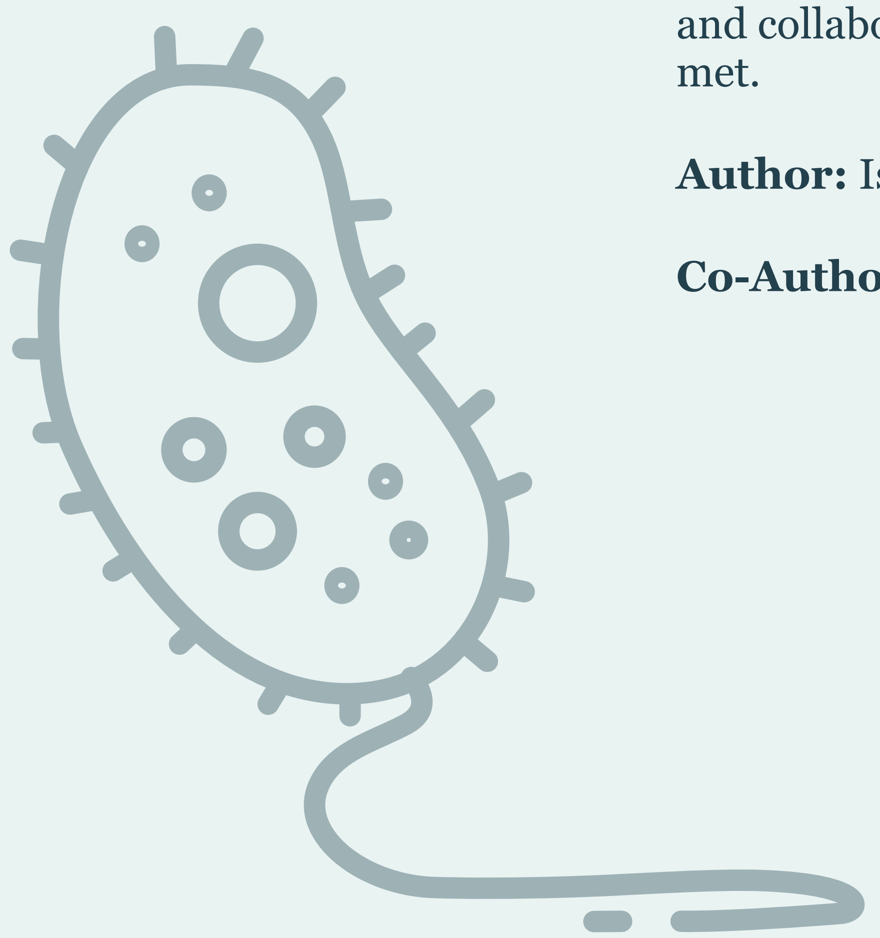
Methods: Qualitative, semi-structured interviews were conducted across four countries with experts from academia, government and environmental regulators, and the wastewater industry. Questions focused on participants' opinions on how both UK environmental regulators and the wastewater industry have responded to the NAP's goals, and their opinions on future policy priorities.

Findings: Most interviewees discussed the topic of "risk." For example, the wastewater industry representatives indicated they would need more evidence showing that wastewater poses a risk of AMR pollution to the environment, in comparison to other pollution sources, in order to implement mitigation strategies. Further, representatives from all industries suggested that more data was needed showing the relative risk of environmental AMR to human health. There were also calls to establish regulatory thresholds for AMR and chemical pollution. Finally, participants felt that communication and collaboration between sectors and UK nations could be improved but that external factors such as Brexit and Covid-19 had taken precedence in recent years and hindered progress.

Implications: To make progress to achieve the environmental AMR NAP goals: mitigations need to be adopted based on the precautionary approach; surveillance markers should be identified for long-term monitoring to establish baselines and inform target baselines; and surveillance should be integrated into existing monitoring networks whilst tailored monitoring is designed and piloted. Further, the relative risk different pollution sources and environmental AMR pose to human health should be further researched and effectively communicated to relevant stakeholders. Finally, establishing formalised networks will allow for enhanced communication and collaboration between different sectors and UK nations to ensure environmental targets are met.

Author: Isobel Stanton, UK Centre for Ecology and Hydrology, United Kingdom

Co-Authors: Tipper, Holly; Pacho, Agata; Glover, Rebecca; Singer, Andrew



Session B

Abstract 32

Poster Board number: 147

Ultra-Accurate Classification and Discovery of Microbial Protein Functions Using FunGeneTyper: An Extensible Deep Learning-Based Framework

Background and Aim: High-throughput DNA sequencing and metagenomics have produced vast quantities of protein-coding gene (PCG) sequences from microbiomes. Decoding the diverse functional (meta)genomic DNA sequences ewithin microbial dark matter is essential. However, accurately assigning protein functions to novel gene sequences remains challenging, particularly for low-homology discovery of functional genes. In this study, we developed an end-to-end, innovative, and extensible deep learning-based framework, FunGeneTyper1, with novel models, structured databases, and new bioinformatics tools for ultra-accurate and fine-grained classification and discovery of functional protein-coding genes.

Procedure/Method: FunGeneTyper implements a two-stage pipeline that separately handles the assignment of the main types and subtypes of PCG functional classes. With tunable adapters, the dual deep-learning model (FunTrans and FunRep) accommodates classification of various PCG types. A lightweight neural network adapter module within FunGeneTyper secures parametrically efficient training.

Findings/Results: The framework achieves ultra-accurate (>0.99) and fine-grained classification and discovery of ARGs and virulence factor genes (VFGs). Using independent ARGs datasets, we confirmed FunGeneTyper's predictive capabilities for low-homology genes. The paradigm and framework can be utilized to develop new plug-and-play neural network lightweight adapters and enables the establishment of an Adapter Sharing Community (ASC). ASC-empowered FunGeneTyper creates an evolving platform, significantly enhancing protein-function prediction accuracy and valuable enzymes discovery, benefiting various fields such as industrial and environmental biotechnology, health, agriculture, and bioenergy.

Implications/Applications: The applicability of our FunGeneTyper framework is clearly not limited to the microbial protein functions (i.e., bacterial antibiotic resistance and virulence) demonstrated in this study and can be conveniently extended to the ultra-accurate classification and discovery of other categories of important protein functions via the adapter module. Our study provides an innovative methodological paradigm -- an extensible deep learning-based framework. FunGeneTyper aims to accurately classify protein functions and unearth valuable enzymes from microbial dark matter, thus advancing microbiome, biotechnology, and bioinformatics fields.

Author: Guoqing Zhang, Westlake University, China

Co-Authors: Ju, Feng

Session B

Abstract 300

Poster Board number: 148

Flash Poster Presentation: S16 - Thursday, May 30 - 8:30-10:15

Genomic analysis of ESBL-producing bacteria recovered from frozen, ready-to-eat, imported stone fruit

Background and Aim: The escalating challenge of antimicrobial resistance (AMR) in infectious bacteria is an urgent public health problem. The role of foodborne bacteria as a potential route for the transmission of AMR is unclear, and current surveillance methodologies target pathogenic species and provide limited information about AMR genes (ARGs) carried by non-pathogenic species commonly found in foods. With Canada's significant reliance on the importation of fresh and frozen produce, this study aims to better understand the Canadian exposure to ARGs from imported, ready-to-eat (RTE), frozen stone fruit.

Procedure/Method: Buffered peptone water enrichments of 161 frozen coconut, mango, avocado, and peach samples were plated on CHROMagar ESBL and MacConkey agar containing cefotaxime to isolate extended spectrum beta lactamase (ESBL)-encoding bacteria of critical concern to public health, particularly those belonging to Enterobacteriaceae. A high-throughput sequencing approach was used to screen over 600 bacterial isolates recovered from these samples to identify clinically relevant ARGs. Isolates carrying ARGs of interest were further characterized by whole-genome sequencing.

Findings/Results: In comparison to other commodities, frozen coconuts contained the highest aerobic bacterial counts pre-enrichment, and bacterial counts on ESBL agars post-enrichment. All of the ESBL-encoding isolates identified originated from frozen coconut samples. Clinically important ARGs, including blaCTX-M, blaSHV, and blaTEM, were identified in 24 different isolates, primarily belonging to the genera Klebsiella, Acinetobacter, Pseudomonas, Enterobacter.

Implications/Applications: These findings provide important information about the occurrence of AMR organisms in imported RTE stone fruits, with frozen coconuts being a particular concern. This study will inform future risk assessments, but also underscores the need for more comprehensive surveillance strategies to mitigate the spread of AMR through global food chains. This work contributes to a broader understanding of the AMR landscape, potentially influencing policy and regulatory measures for food safety and public health protection.

Author: Nasta, Adeeb, Catherine Carrillo, Canadian Food Inspection Agency (CFIA) - Ottawa Laboratory Carling, Canada

Co-Authors: Cooper, Ashley; Tamber, Sandeep

Session B

Abstract 159

Poster Board number: 149

Mining "Big Text" on Antimicrobial Resistance with Natural Language Processing

Background and Aim: Increasing antimicrobial resistance (AMR) is a global health crisis, causing a substantial healthcare and socioeconomic burden. However, better methods are needed to integrate the knowledge from numerous AMR publications. Herein we provided a powerful AI-based tool to mine text and data in AMR literature by Natural Language Processing (NLP). Our goal was to characterize AMR research globally and to guide and inform AMR policy- and decision-making making.

Procedure/Method: We constructed a database containing 254,847 AMR-related publications published over the past two decades. We parsed the abstracts and extracted the words and noun phrases by NLP-based methods. Named Entity Recognition (NER) was utilized to match the literature with corresponding conducted countries. The Inverted Index was employed to linearly optimize the efficiency of complex information acquisition. We identified previous research foci and current knowledge gaps including specific pathogens, pathogen-drug combinations, antibiotic resistance genes (ARGs), and existing AMR surveillance methodologies across regions and income levels.

Findings/Results: Our methodology was efficient and robust in distilling knowledge from massive available literature. Our NLP-based model performed AMR categorizing accuracy of 97.4% and took about 0.6 microseconds to extract all related literature for one specific topic. We showed the dominant approaches for studying AMR different pathogens and ARGs of study across countries. The most common pathogen-drug combinations studied were methicillin-resistant S. aureus, ESBL-producing E. coli, and carbapenem-resistant Enterobacteriaceae, and dominants ARGs were mecA, gyrA, and ampC. Research efforts are impressive, but broadly lack publications from low-income countries (LICs), which is a huge knowledge gap.

Implications/Applications: We constructed a powerful AI-based methodology to integrate knowledge from massive literature, and this analysis is the first to synthesize and map global AMR research patterns by NLP. Our methodology can be used to develop, disseminate, and create new tools and practices for sharing knowledge and information on AMR.

Author: Shule Li, Institute of Urban Environment, Chinese Academy of Sciences, China

Co-Authors: Chen, Cai; Zhu, Yong-guan; Graham, David

Back to the Program

Session B

Abstract 268

Poster Board number: 150

Flash Poster Presentation: S11 - Tuesday, May 28 - 13:45-15:30

Factors influencing horizontal dissemination of antibiotic resistance genes

Background and Aim: The dissemination of mobile antibiotic resistance genes (ARGs) among bacterial strains and species via horizontal gene transfer is a key component of the growing antibiotic resistance crisis. However, the extent to which different genetic and environmental factors impact this gene flow remains unknown. In this study, we aim to study how genes encoding different resistance mechanisms have spread between distantly related bacteria and identify factors that are associated with the successful horizontal transfer of ARGs.

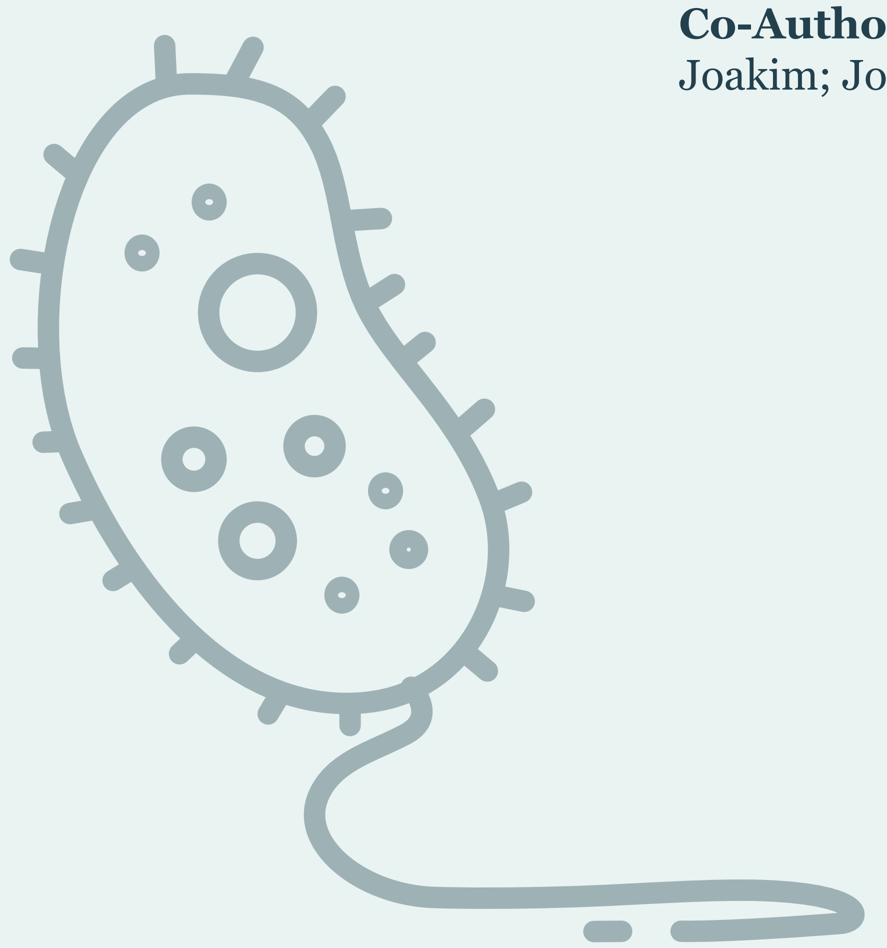
Method: Based on a collection of 60,796 unique ARGs predicted in 867,318 bacterial genomes, we used phylogenetic analysis to identify 6,276 instances of highly similar genes being carried by evolutionarily distant bacterial hosts, which were inferred as horizontal transfers. To identify factors influencing these transfers, features describing the genetic compatibility of the involved gene(s) and genomes, the co-occurrence of the involved bacteria in different environments (based on 24,417 metagenomic samples), and the cell wall structure of the involved species were collected for each observed transfer and used as input to train random forest classifiers.

Results: Our resulting models were able to reliably identify bacterial host pairs associated with horizontally transferred ARGs (mean AUC > 0.82). The models further suggested that ARGs are more likely to be horizontally transferred between bacteria whose genomes have a similar nucleotide composition, and whose cell walls have the same structure in terms of Gram staining. Moreover, we found that horizontal dissemination of ARGs was most frequently observed between species that colonize human and wastewater microbiomes.

Implications: This study, to our knowledge, marks the broadest attempt to quantify the impact of different factors on the horizontal dissemination of ARGs. This information could be vital for implementing management strategies to deal with the antibiotic resistance crisis.

Author: David Lund, Chalmers University of Technology, Sweden

Co-Authors: Parras-Moltó, Marcos; Inda-Díaz, Juan S.; Ebmeyer, Stefan; Larsson, D. G. Joakim; Johnning, Anna; Kristiansson, Erik



Session B

Abstract 284

Poster Board number: 151

Flash Poster Presentation: S11 - Tuesday, May 28 - 13:45-15:30

Resistance Gene Identifier (RGI) - Prediction of antimicrobial resistance genes in metagenomic sequencing data

Background: The determinants of antimicrobial resistance (AMR) are at the center of health and disease in the biosphere. There is evidence that the AMR genes spread from one environment to the next due to pressures of microbial competition and anti-infective usage. There is a need to keep track of AMR determinants that pose significant health risks to all aspects of one health as a whole. There is rapid adoption of next-generation sequencing in both people and animal health, thus we can leverage these data for AMR surveillance by developing prediction software that uses reference data that is continuously updated in the AMR context. We developed the Resistance Gene Identifier (RGI) software which uses the actively maintained reference database the Comprehensive Antibiotic Resistance Database (CARD; https://card.mcmaster.ca) to predict AMR determinants.

Methods: We used metagenomic samples from soil and clinic that are enriched and whole genome sequencing (WGS). The reads were aligned to CARD canonical and CARD variants data using RGI's metagenome module called RGI bwt to predict AMR determinants in the samples. All the read data went through quality checks i.e. trimming, removing duplicates, and removing low-quality reads.

Results: The RGI bwt can predict the same AMR determinants before and after de novo assembly. The RGI bwt can also predict AMR determinants with low-quality data but more CARD variants are needed to map reads for WGS as compared to the targeted capture or enriched.

Conclusion: The RGI bwt provides an option to skip assembly, which makes it feasible to process large amounts of data to predict AMR determinants quickly. Mining AMR genes from the GenBank to produce CARD variants is an important step, but this only makes us aware of clinical AMR and less of the environment. RGI bwt is suited to be used for AMR surveillance.

Author: Amogelang Raphenya, McMaster University, Canada

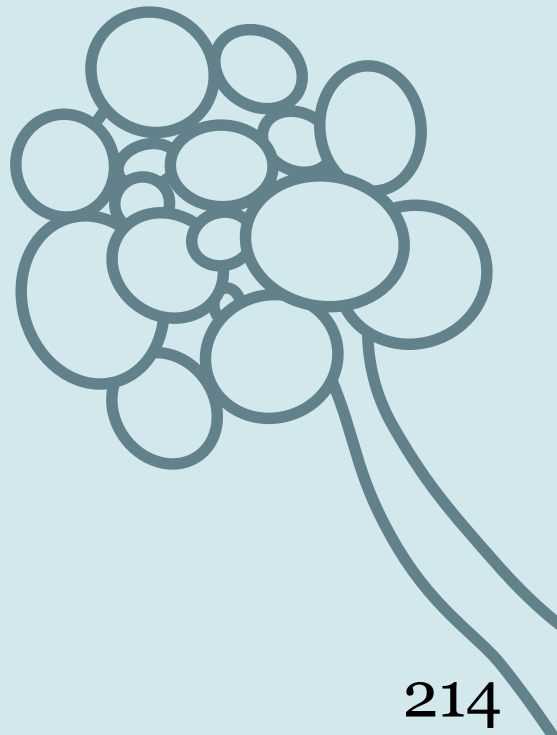
Co-Author: Alcock, Brian; Hackenberger, Dirk; McArthur, Andrew

Exhibition Plan

1	AMR Center
2	Illumina
3	QIAGEN Inc.
4	Resistomap
5	PacBio
6	Aquagenx
7	Génome Québec
8	Public Health Agency of Canada

‖ Poster board

🍴 Food and Beverages



AQUACULTURE
WATER & WASTEWATER
PUBLIC HEALTH
RESEARCH
FOOD SAFETY
LIVESTOCK
ANTIBIOTIC USE

Genome Alberta

GENOMICS AT WORK FOR

ACTION ON ANTIMICROBIAL RESISTANCE

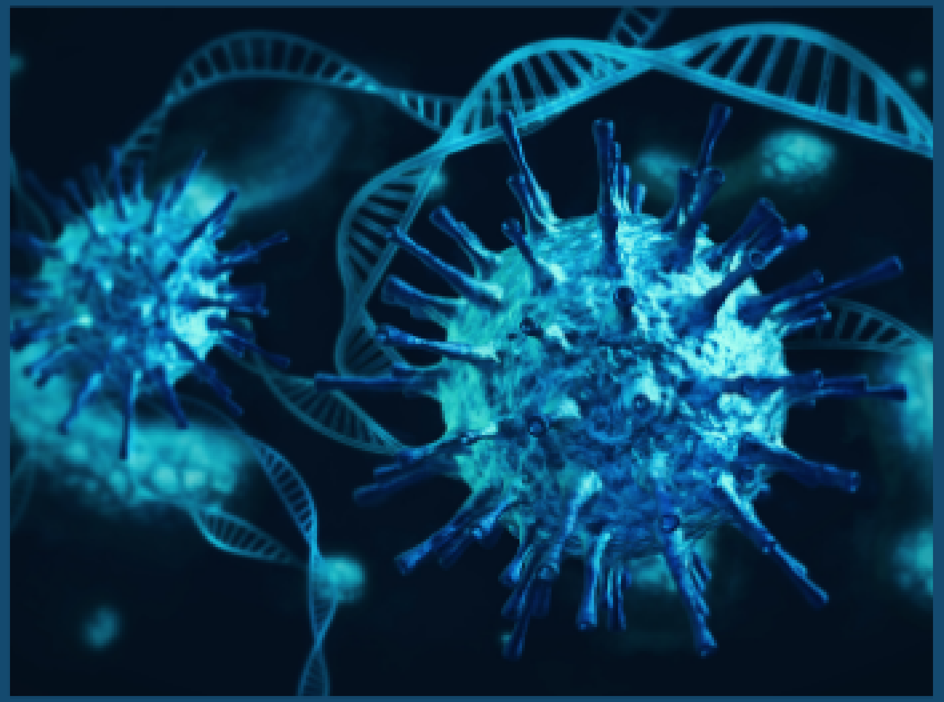
See genomealberta.ca for funding information.

GenomeQuébec

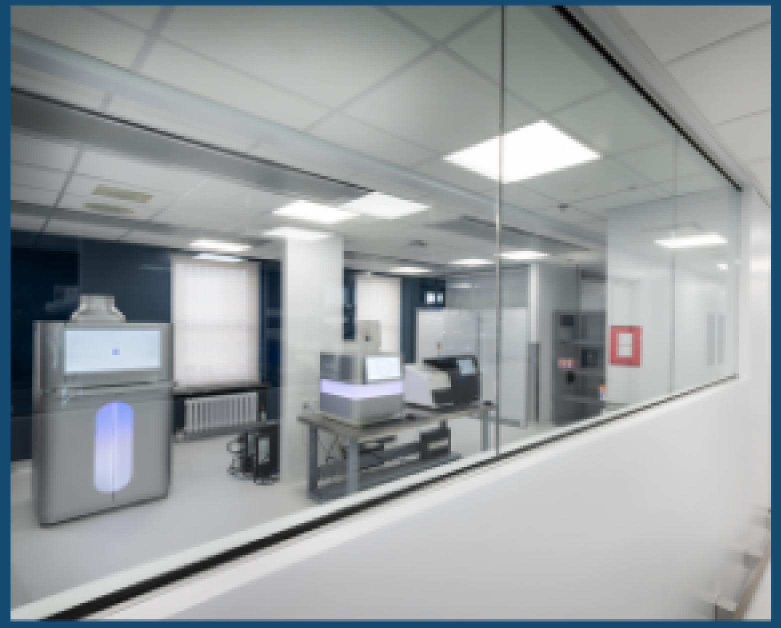
Our mission is to catalyze the development and excellence of genomics research, its integration and democratization.

In 2023, Génome Québec launched a major funding opportunity to help researchers combat antimicrobial resistance. Six research teams were selected, representing a total investment of over $4.5 million. These projects will investigate novel infections, identify emerging pathogens, and develop new tools for the surveillance of AMR. Scan the code below to read more about the funded projects and how Génome Québec is contributing to this rising global challenge.

Learn more about the projects



Centre d’expertise et de services Génome Québec



With a solid team of highly qualified technicians and managers, our centre supports teams of academic and industrial researchers in over 50 countries. We offer a wide range of services to suits your needs:

- Next generation sequencing
- Long read sequencing
- Extraction from biological samples
- Sanger sequencing
- Genotyping
- Analysis of structural variations
- Biobanking

For more information about our services, visit us at genomequebec.com