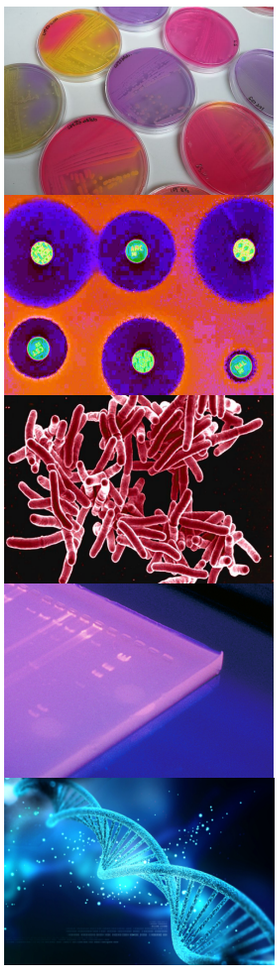
Characterization and quantification of antibiotic resistance gene variants in gut microbiota

Ouléye Sidibe, Anne-Carmen Sanchez, Guillaume Kon Kam King, Fanny Calenge, Benoît Doublet, Sylvie Baucheron, Anne-Laure Abraham, Sébastien Leclercq



Abstracts book

9th Symposium on Antimicrobial Resistance in Animals and the Environment

2023

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Contents

ARAE 2023: An introduction

The emergence of antimicrobial resistance is a seminally important public health concern. Significant progress has been made in recent years regarding an understanding of the genetic and biochemical basis for antimicrobial resistance, the emergence of resistance genes, and factors promoting their widespread dissemination including the role of lateral gene transfer. Nevertheless, there is a dearth of information regarding the key 'hotspots' and genetic mechanisms responsible for resistance development, and the exposure routes leading to the failure of antimicrobial agents important in human and animal medicine. There is thus an urgent need for research to provide governments, public health stakeholders, and the agricultural sector the knowledge required to develop policies and practices that effectively mitigate resistance development. This, within a growing recognition that humans, animals and the environment must be considered as intimately linked together if any resistance management strategy is to be successful.

Livestock are in close contact with soil and water, natural reservoirs of microbiota harbouring resistance genes. In turn, the use of manures as fertilizers for crop production is a potentially important source for environmental contamination of resistance genes selected for and enriched in the animal. A better understanding of the significance of animal and environmental reservoirs of antimicrobial resistance, and factors leading to the emergence and dissemination of antibiotic-resistant bacteria in agricultural production systems is a priority.

The ARAE symposium, created by scientists from INRAE, is organized since 2005. Since the first edition in Lyon, ARAE has been a great success with many scientists from all over the world still participating in this symposium which is now in its ninth edition.

Benoît Doublet	Axel Cloeckaert	Michel S. Zygmunt
Chair	*Co-Chair*	*Co-Chair*

Scope and aim of ARAE 2023

The aim of the ARAE conference is to present a global vision of the impact of antibiotic use and resistance in the animal world, its environment and consecutive repercussion on human health. During six sessions, all aspects related to epidemiology of antibiotic-resistant bacterial pathogens with a zoonotic potential, mobile elements containing resistance genes, emerging antimicrobial resistance mechanisms, resistome of microbiotas, and the role of the environment as dissemination routes and potential source of resistance genes transfer will be discussed.

Themes

1- Roles of the environment in resistance evolution and transmission

While acquisition of new resistance factors in pathogens is uncommon, acquisition of resistance factors from other microbial species may occur in diverse natural microbial ecosystems, and may impact the prevention and cure of bacterial infections both in humans and animals. On the other hand, environmental pollution by already widespread resistances is common. Therefore, environmental health (water, sanitation, waste management, food hygiene and safety) appears essential to prevent the spread of antibiotic-resistant bacteria and reduce the global antibiotic resistance threat.
This session will consider the source, propagation, transfer and dissemination of resistance genes in the environment (aquatic, terrestrial, …). The impact of agricultural practices, such as the veterinary use of antimicrobial agents and use of fertilizers of animal and human origins, will be emphasized.

2- Monitoring and molecular epidemiology of antimicrobial resistance

To determine the prevalence of antimicrobial resistance among the most relevant veterinary and zoonotic pathogens, monitoring programs are currently conducted worldwide either as large-scale national monitoring programs or as drug- specific monitoring studies. The results of such monitoring programs – in particular when supported by (a) molecular analysis of the resistance genes and resistance-mediating mutations, (b) studies on the transferability of the resistance properties, (c) genomic analysis of resistant strains, and (d) consumption data of antimicrobial agents – provide important information for the understanding of the development and spread of antimicrobial resistance. Examples of the results of such monitoring programs highlighting the molecular epidemiology of resistance properties will be presented and discussed in this session.

3- Mechanisms and dissemination of antimicrobial resistance in animal and zoonotic pathogens

Gaining insight into the mechanisms of antimicrobial resistance, long-term persistence, and successful spread, is fundamental to reduce the burden of antimicrobial resistance. This session will focus on emerging antimicrobial resistance mechanisms in animal and zoonotic pathogens. All mechanisms will be considered such as target modification, protection of the target, efflux, decreased permeability, enzymatic inactivation as well as induction of resistance mutagenesis... All mechanisms implicated in the successful spread and persistence of mobile genetic elements harbouring resistance genes (plasmids, integrative elements,...) will be also of particular interest for this session.

4- Understanding the connection of antimicrobial resistance between Animals and Humans

Antimicrobials used to treat infectious diseases in animals are often the same or similar to those used to treat human infections. Antibiotic-resistant bacteria may spread between animals and humans and reciprocally, and through their environment as well. It may in turn result in worldwide epidemics of

antibiotic resistant genes and their vehicle.
The spread of antibiotic-resistant bacteria and genes across ecosystems can occur through multiple ways, agriculture-related such as reuse of animal wastewater and manures, via the food chain (zoonotic or animal food contamination) and also by direct contact between animal and human (companion animals and wild life).
This session aims to gain insight on the origin and dissemination routes of antibiotic-resistant bacteria and genes in the animal-human-environment continuum. We welcome presentations providing new knowledge and insight in this field.

5- Novel approaches, methods and tools dedicated to antimicrobial resistance (detection, evolution, diagnostics, surveillance)

Monitoring antibiotic resistance dissemination in husbandry or in the environment, investigating the links between antimicrobial resistance in animals and humans, or exploring the diversity of mechanisms of resistance and mobile genetic elements, all rely on the production of an ever-growing amount of data. Specific tools and methodologies designed to manage such data are therefore constantly developed. The session is open for description of new methods and their diverse applications for exploring or monitoring antimicrobial resistance. Tools for genomic or metagenomic analyses as well as original molecular approaches, modeling approaches or laboratory/sampling procedures are all welcome in this session.

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Symposium Venue

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Monday July 3

08h00-08h50 Reception/Registration
08h50-09h00 Symposium opening

Session 1 - Roles of the environment in resistance evolution and transmission

Chair: **Sabine Favre-Bonté**
Co-chair & keynote speaker: **Ashley SHADE**

09h00-09h40: **Ashley SHADE**
Antibiotic resistance and production genes in the soil microbiome given anthropogenic disturbance: insights from the Centralia coal seam fire

09h40-09h55: **Ashlyn H. STRICKLAND**, Sarah A. MURRAY, Javier VINASCO, Jason E. SAWYER, Harvey M. SCOTT, Brent. W. AUVERMANN, Jack BUSH, and Keri N. NORMAN
Metagenomic analysis of the bacterial community structure within cattle feces, the feedyard environment, and particulate matter as a function of antibiotics, probiotics, and environmental manipulation

09h55-10h10: Gonçalo MACEDO, **Joost HORDIJK**, Pieter VAN VEELEN, Lucia HERNANDEZ LEAL, Alex BOSSERS, Asmus Kalckar OLESEN, Søren J. SØRENSEN, Dik HEEDERIK, Dik MEVIUS, and Heike SCHMITT
The effect of manure amendment on the soil grassland resistome

10h10-10h25: **Michaela RUZICKOVA**, Kristina NESPOROVA, Jana PALKOVICOVA, Simon KREJCI, Ivan LITERAK, and Monika DOLEJSKA
Dynamics of cephalosporin-resistant in *Escherichia coli* in gulls highlight the importance of longitudinal wildlife studies

10h25-10h40: **Dayana JARMA**, Oriol SACRISTÁN-SORIANO, Francisco HORTAS, Juan M. PERALTA-SÁNCHEZ, Alexandre SÁNCHEZ-MELSIÓ, José L. BALCÁZAR, Carles M. BORREGO, and Marta I. SÁNCHEZ
Spatial spreading of antibiotic resistance genes by gulls

10h40-11h10: Coffee break/Poster

11h10-11h25: Charlène SAGRILLO, Christophe MERLIN, and **Xavier BELLANGER**
Compartmentalization of antibiotic resistance between phages and bacteria in a river - wastewater treatment plant continuum

11h25-11h40: **Caroline WYBRANIEC**, Geneviève GAGNE, Cécile MOUSSARD, Marion BEAUPERE, Léa LUSURIER, Françoise LERICHE, Laurence MARJOLET1, Nicolas SERTILLANGES, Dominique PATUREAU, Benoit COURNOYER, and Wessam GALIA
Occurrence of 41 sanitary indicators in digestates derived from different anaerobic digestion processes and various raw organic wastes

11h40-11h55: **Brune JOANNARD**, Timothy M. VOGEL, and Concepcion SANCHEZ-CID
Microplastics increase the selective potential of antibiotics at sub-inhibitory concentrations

11h55-12h10: **Federica PIERGIACOMO**, Franck LEJZEROWICZ, Tam TRAN, Atif A. CHOWDURY, Luciano BENEDUCE, Odd-Gunnar WIKMARK, Leonardo PAGANI, Alexander EILER, and Lorenzo BRUSETTI
Do microplastics offer a suitable surface for antibiotic resistance spread into the open environment?

12h10-12h25: **Florent ROSSI**, Eloïse LEBRAS, Eric DEFERT, Nathalie TURGEON, Marc VEILLETTE, Jean-Luc BARAY, Pierre AMATO, and Caroline DUCHAINE
Can antimicrobial resistance genes travel across the Atlantic Ocean?

12h30-14h00: Lunch

Session 2 - Monitoring and molecular epidemiology of antimicrobial resistance

Chair: **Jean-Yves MADEC**
Co-chair & keynote speaker: **Muna ANJUM**

14h00-14h40: **Muna ANJUM**
Use of genomics to characterise antimicrobial resistances present within livestock on UK farms

14h40-14h55: **Olgica CERIC**, Jake GUAG, Sarah PELOQUIN, Amy MERRILL, Chih-Hao HSU, Claudine KABERA, and Gregory H. TYSON
The role of FDA's Veterinary Laboratory investigation and response network in monitoring antimicrobial resistance of animal pathogens

14h55-15h10: **Henrike KRÜGER-HAKER**, Xing JI, Dennis HANKE, Stefan FIEDLER, Andrea T. FEßLER, Nansong JIANG, Heike KASPAR, Yang WANG, Congming WU, and Stefan SCHWARZ
Genomic diversity of porcine LA-MRSA CC398 isolates collected in the German national resistance monitoring program GE*RM*-Vet between 2007 and 2019

15h10-15h25: **Janine MULLER**, Yuhong LIU, Ilhan MOHAMMAD, Brendan RODONI, and Fiona CONSTABLE
Antimicrobial resistance in pathogenic bacteria of Victorian cattle

15h25-15h40: **Maud de LAGARDE**, John M. FAIRBROTHER, Marie ARCHAMBAULT, Simon DUFOUR, David FRANCOZ, Jonathan MASSÉ, and Jean-Philippe ROY
Clonal and plasmidic dissemination of critical antimicrobial resistance genes in dairy cattle in Québec, Canada

15h40-15h55: **Virginie GUÉRIN**, Nicolas CABANEL, Damien THIRY, Jacques MAINIL, Marc SAULMONT, and Philippe GLASER
A phylogeny study of ß-lactam-resistant *E. coli* from Belgian calves

15h55-16h05: **Amelie BARTHELEMY** (StandardBio Tools)
Setting the new benchmarks in automated and cost-effective qPCR and NGS assay using a single platform

16h10-16h40: Coffee break/Poster

16h45-17h00: **Ahmad I. AL-MUSTAPHA**, Emmanuel AJ. AWOSANYA, Victoria O. ADETUNJI, and Annamari HEIKINHEIMO
Multidrug-resistant *Escherichia coli* isolated from slaughtered broilers in Ilorin, Nigeria

17h00-17h15: **Moniek RINGENIER**, Filip BOYEN, Sigrid C.J. DE KEERSMAECKER, Kevin VANNESTE, Bert BOGAERTS, Mathias DEVREESE, and Jeroen DEWULF
Fluoroquinolone resistance in *E. coli* in broilers: mapping the spread

17h15-17h30: **Alicia MANZANARES**, Florencia CORREA-FIZ, Teresa AYATS, Miquel NOFRARÍAS, and Marta CERDÀ-CUÉLLAR

In-vitro validation of AMR genes prevalence revealed by WGS in *Campylobacter jejuni* isolated from chicken livers in Spain

17h30-17h45: Ojas DIXIT, Aidan PREUSS, and **Claire L O'BRIEN**
Antimicrobial resistance determinants in bacteria isolated from retail chicken and pork meat across New South Wales, Australia

17h45-18h00: **Ramon P. MALUPING**, Remedios F. MICU, Thomas CHISNALL, Alistair DAVIES, Evelyn E. EMBESTRO, Mary Ann ESCOTO, Mildred A. PADILLA, and Roderick CARD
Prevalence of Extended-Spectrum Beta-Lactam (ESBL) and colistin resistance in *Escherichia coli* isolated from broiler chickens in dressing plants in the Philippines

18h00-18h15: Joel JAVID, Emmanuelle MOREAU, Lionel PINNEAU, Niki HAYATGHEIB, Claire MALTRET, Segolene CALVEZ, and **Nora NAVARRO-GONZALEZ**
Risk factors for antimicrobial resistant *Aeromonas* spp. and *Escherichia coli* in the rainbow trout farm environment in Brittany, NW France

18h15-19h00: Poster session

19h00: Welcome reception

Tuesday July 4

Session 2 - continued

Chair: **Jean-Yves MADEC**
Co-chair & keynote speaker: **Muna ANJUM**

09h00-09h40: **Windi Muziasari**
Abundance and diversity of antibiotic resistance genes in different types of environmental samples: 5 years of Resistomap

09h40-9h55: **Lorcan O'NEILL**, Maria RODRIGUES da COSTA, Edgar GARCIA MANZANILLA, Fiona CRISPIE, Raul CABRERA-RUBIO, Rachel GRIMES, and Finola C. LEONARD[1]
The evolution of the faecal resistome is shaped by age and antimicrobial use during the pig lifecycle

09h55-10h10: **Mike BROUWER**, Alieda van ESSEN-ZANDBERGEN, Yvon GEURTS, Frank HARDERS, and Kees VELDMAN
Genetic analysis of ampC promotor mutants of *E. coli* from livestock exhibiting resistance to extended-spectrum cephalosporins

10h10-10h25: **Sabine DELANNOY**, Corine HOFFER, Raphaëlle YOUF, Emilie DAUVERGNE, Hattie E. WEBB, Thomas BRAUGE, Mai-Lan TRAN, Graziella MIDELET, Sophie A. GRANIER, Marisa HAENNI, Patrick FACH, and Anne BRISABOIS
High throughput screening of antimicrobial resistance genes in Gram-negative seafood bacteria

10h25-10h40: **Sandra MARTÍNEZ-ÁLVAREZ**, Pierre CHÂTRE, Úrsula HÖFLE, Carla Andrea ALONSO, Pauline FRANÇOIS, Teresa CARDONA-CABRERA, Myriam ZARAZAGA, Jean-Yves MADEC, Marisa HAENNI, and Carmen TORRES
High occurrence of carbapenemase- and extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from migratory birds (*Ciconia ciconia*) with detection of high-risk clones

10h45-11h15: Coffee break/Poster

11h10-11h25: **Maeve Louise FARRELL**, Georgios MILIOTIS, Firinne KELLY, Evan NAUGHTON, Brigid HOOBAN, Alexandra CHUEIRI, Mark MAGUIRE, Louise O' CONNOR, Martin CORMICAN, Niamh CAHILL, Kelly FITZHENRY, Aoife JOYCE, Sinead DUANE, Liam P. BURKE, and Dearbhaile MORRIS
Assessing the resistome and virulome of human and environmental *Escherichia coli* isolates

11h25-11h40: **Camille FAVIER**, Mylène TOUBIANA, Olivier COUILLEROT, Elodie PICHON, Patricia LICZNAR-FAJARDO, and Estelle JUMAS-BILAK
Wastewater-based Epidemiology (WBE): toward a relevant tool to monitor hospital antimicrobial resistance

11h40-11h55: **Andrea OTTESEN**, Brandon KOCUREK, Shawn BEHLING, Claudine KABERA, Patrick MCDERMOTT, and Errol STRAIN
Metagenomic survey of antimicrobial resistance in surface waters of Maryland with high and low human impact classifications

11h55-12h10: Iva SUKKAR, Adam VALCEK, Martin KLVANA, Jarmila LAUSOVA, Kristina NESPOROVA, **Jana PALKOVICOVA**, and Monika DOLEJSKA
Gram-negative bacteria resistant to last-line antibiotics from hospital and municipal wastewaters

12h10-12h25: **Mélanie PIMENTA**, Degrâce BATANTOU, Margaux GASCHET, Stéphanie GUYOMARD, Marie-Cécile PLOY, Sébastien BREUREC, and Christophe DAGOT on behalf of the ANR ACRAS-R consortium
One-Health AMR spread in a French Caribbean island

12h30-14h00: Lunch

Session 3 - Mechanisms and dissemination of antimicrobial resistance in animal and zoonotic pathogens

Chair: **Axel CLOECKAERT**
Co-chair & keynote speaker: **Séamus FANNING**

14h00-14h40: Séamus FANNING
Klebsiella a hitherto underappreciated zoonotic pathogen expressing AMR phenotypes of importance to One Health

14h40-14h55: **Valentina DONÀ**, Sonja KITTL, Andrea ENDIMIANI, and Vincent PERRETEN
WGS-based characterization of emerging pathogenic OXA-48-producing *Enterobacter hormaechei* belonging to ST114 and ST418 in a Swiss companion animal clinic

14h55-15h10: **Jana PALKOVICOVA**, Adam VALCEK, and Monika DOLEJSKA
Massive plasticity of IncHI2-ST3 plasmids encoding clinically important IMP-4 in bacteria from silver gulls

15h10-15h25: **Laurent POIREL**, Otávio Hallal FERREIRA RARO, and Patrice NORDMANN
Determining the impact of veterinary antibiotics sub dosage on resistance transfer in animal gut microbiota - a One-Health approach

15h25-15h40: Suad ALGARNI, Jing HAN, Dereje GUDETA, Rajesh NAYAK, and **Steven L. FOLEY**
In silico analyses of Incompatibility group (Inc) HI2 plasmids from enteric bacteria

15h40-15h55: **Indre NAVICKAITE**, Harry HOLMES, Letizia DONDI, Luke RANDALL, Catherine FEARNLEY, Emma TAYLOR, Edward FULLICK, Manal ABUOUN, Christopher TEALE, and Muna F. ANJUM
Investigation of *rmtB*-harbouring *Salmonella* and *Escherichia coli* from a pig farm

16h00-16h30: Coffee break

16h35-16h50: Xingyang DAI, Li CHEN, Junjie SUN, Jinhu HUANG, and **Liping WANG**
Various mobile genetic elements involved in the dissemination of phenicol-oxazolidinone resistance gene *optrA* in *Streptococcus suis*

16h50-17h05: Seiji YAMASAKI, Ryosuke NAKASHIMA, Takayoshi SUZUKI, and **Kunihiko NISHINO**
Development of efflux pump MexB and MexY dual inhibitors against multi-drug resistant *Pseudomonas aeruginosa*

17h05-17h20: **Katie WALL**, Leonard KOOLMAN, Guerrino MACORI, and Séamus FANNING
Glyphosate induces changes to cellular metabolic pathways and selects for antimicrobial resistance in *Klebsiella* species

17h20-17h35: **Amandine CHAUVIAT**, Gwenaëlle GRAULIER, Sylvie NAZARET, and Sabine FAVRE-BONTE

Quinolone resistance and expression of RND efflux pump in *Stenotrophomonas* spp. under exposure to sub-lethal concentration of nickel

17h35-17h55: **Emilie DEHON**, Alban MATHIEU, Arnaud DROIT, Timothy M. VOGEL, and Concepcion SANCHEZ-CID
Heavy metals may increase ARG persistence in the environment

20h00-Midnight: Gala dinner

Wednesday July 5

Session 4 - Understanding the connection of antimicrobial resistance between Animals and Humans

Chair: **Laurent POIREL**
Co-chair & keynote speaker: **Vincent PERRETEN**

09h00-09h40: **Vincent PERRETEN**
WGS-based relationship between multidrug-resistant bacteria from humans and animals: emphasis on CPE and MRSA

09h40-9h55: **Rajesh NAYAK**, Jing HAN, and Steven L. FOLEY
U.S. FDA's National Center for toxicological research AMR research: applications across the One Health Continuum

09h55-10h10: **Juliette HAYER**, Ella MARCY, Mallorie HIDÉ, Savatey HAK, Sivhour CHIEK, Navin SRENG, Meymey LEM, Rina DORK, Chiva MA, Gauthier DELVALLEZ, Patrice PIOLA, Véronique CHEVALIER, Anne-Laure BAÑULS, and Sokleaph CHENG
Investigating the circulation of AMR between animals, humans and their environment in Cambodia - ARCAHE

10h10-10h25: Sakib RAHMAN, and **Aidan HOLLIS**
The effect of antibiotic usage on resistance in humans and food-producing animals: a longitudinal, one-health study using European data

10h30-11h00: Coffee break/Poster

11h05-11h20: **Michaela RUZICKOVA**, Ivana JAMBOROVA, Tomas NOHEJL, Iva SUKKAR, Jana PALKOVICOVA, Ivo PAPOUSEK, Max CUMMINS, Steven DJORDJEVIC, and Monika DOLEJSKA
Comparative genomics of *Escherichia coli* ST131 of human, animal and environmental origin from the Czech Republic

11h20-11h35: Joyshri SARKER, Aylar SABA SHIRVAN, Céline DAVID, Stephane CORVEC, Eric BATARD, Maria UGARTE-RUIZ, Ségolène CALVEZ, and **Nora NAVARRO-GONZALEZ**
Understanding the risks associated with recirculating aquaculture systems: persistence of antimicrobial-resistant *Enterococcus* spp. and comparison with isolates from human infections

11h35-11h50: **Marta ROZWANDOWICZ**, Henrik HASMAN, Mike SM BROUWER, Muna F ANJUM, Stefan BÖRJESSON, Manuela CANIÇA, Jens A HAMMERL, Virginia CARFORA, Benoît DOUBLET, Antoni PA HENDRICKX, Emma ÖSTLUND, Jannice S SLETTEMEÅS, Magdalena ZAJĄC, Pieter-Jan CEYSSENS, Joost HORDIJK, and the Full Force consortium
Successful host adaptation of IncK2 plasmids

11h50-12h05: **Ulrike BINSKER**, Kathrin OELGESCHLÄGER, Annemarie KÄSBOHRER, and Jens A. HAMMERL
Indications of transmission of *mcr*-1.26 IncX4 plasmids along the poultry food chain to humans

12h10-13h40: Lunch

Session 5 - Novel approaches, methods and tools dedicated to antimicrobial resistance (detection, evolution, diagnostics, surveillance)

Chair: **Sébastien LECLERCQ**
Co-chair & keynote speaker: Etienne RUPPE

13h40-14h20: **Etienne RUPPE**
The present and future in antimicrobial resistance surveillance

14h20-14h35: **Carole KOWALEWICZ**, Michael TIMMERMANS, David FRETIN, Pierre WATTIAU, and Cécile BOLAND
An in-house 45-plex array for the detection of antimicrobial resistance genes in Gram-positive bacteria

14h35-14h50: **Yolanda GONZALEZ-FLORES**, Margaux GASCHET, Mélanie PIMENTA, Christophe DAGOT, and Marie-Cécile PLOY
Identification of novel antibiotic resistance integron gene cassettes in water samples with different anthropogenic levels

14h50-15h05: **Samuel J. BLOOMFIELD**, Aldert L. ZOMER, Justin O'GRADY, Gemma L. KAY, John WAIN, Nicol JANECKO, Raphaëlle PALAU, and Alison E. MATHER
Determination and quantification of microbial communities and antimicrobial resistance on food through host DNA-depleted metagenomics

15h05-15h20: **Ouléye SIDIBE**, Anne-Carmen SANCHEZ, Guillaume KON-KAM-KING, Fanny CALENGE, Benoit DOUBLET, Sylvie BAUCHERON, Sébastien LECLERCQ, and Anne-Laure ABRAHAM
Characterization and quantification of antibiotic resistance gene variants in the gut microbiota

15h20-15h35: **Sarah NAUDIN**, Aude FERRAN, Pedro IMAZAKI, Nathalie ARPAILLANGE, Camille MARCUZZO, Maïna VIENNE, Sofia DEMMOU, Felipe RAMON-PORTUGAL, Marlène LACROIX, Claire HOEDE, Maialen BARRET, Véronique DUPOUY, Delphine BIBBAL
Development of an in vitro biofilm model for the study of the impact of fluoroquinolones on sewer biofilm microbiota

15h35-15h50: **Athina ANDREA**, Susanne KARLSMOSE PEDERSEN, Lauge HOLM SØRENSEN, Jette SEJER KJELDGAARD, and Rene S. HENDRIKSEN
From genotype to antimicrobial resistance (AMR) phenotype prediction: how genomic proficiency tests can facilitate the AMR laboratories' performance evaluation

15h50-16h05: **Miki IKEBE**, Kota AOKI, Mitsuko HAYASHI-NISHINO, and Kunihiko NISHINO
Correlation analysis of bacterial cell morphology and multidrug resistance

16h10-16h25: Awards for the best oral and poster presentations

and Symposium closure

Session 1 - Roles of the environment in resistance evolution and transmission

O1

Antibiotic resistance and production genes in the soil microbiome given anthropogenic disturbance: insights from the Centralia coal seam fire

Ashley Shade

CNRS, Université Lyon, University Claude Bernard Lyon 1, Ecologie Microbienne

Environmental disturbances can affect the diversity and persistence of microbial antibiotic resistance and production genes. We leveraged the unique, seven-decade-old underground coal seam fire ongoing in Centralia, Pennsylvania, USA, to examine surface soil bacterial microbiome response to an unnatural, long-term heat disturbance. As the fire slowly advances along coal seams, formerly fire-affected soils cool to ambient temperature enabling geographically localized comparisons of impacted soils across a gradient of fire intensities with recovered and reference soils. We executed a seven-year field study, annually sampling sites selected to capture the range of disturbance intensities. We used amplicon gene sequencing to assess changes in bacterial microbiome structure in response to the fire and untargeted metagenome sequencing to assess changes in the soil functional potential, including assessment of antibiotic resistance and production. Over the years, the fire-affected sites cooled, and their microbiome structures recovered towards those of reference and recovered soils. We present unpublished data on the diversity and patterns of antibiotic resistance and production genes over time and with fire cooling in the impacted sites compared to the ambient-temperature sites. This work contributes to understanding how disturbance affects the functional potential of environmental antibiotic resistance and production.

O2

Metagenomic analysis of the bacterial community structure within cattle feces, the feedyard environment, and particulate matter as a function of antibiotics, probiotics, and environmental manipulation

Ashlyn H. Strickland[1], Sarah A. Murray[2], Javier Vinasco[2], Jason E. Sawyer[3], Harvey M. Scott[2], Brent. W. Auvermann[4], Jack Bush[4], and Keri N. Norman[1]

[1]*Department of Veterinary Integrative Biosciences, Texas A&M University, College Station, TX, USA*
[2]*Department of Veterinary Pathobiology, Texas A&M University, College Station, TX, USA.*
[3]*Department of Animal Sciences, Texas A&M University, College Station, TX, USA*
[4] *Texas A&M AgriLife Research, Amarillo, TX, USA*

The cattle feedyard environment has been shown to harbor antimicrobial resistant (AMR) bacteria and is implicated as a reservoir capable of spreading AMR to nearby animals and humans via effluent water, airborne particulate matter, and manure [1]. Multiple studies have investigated the potential for airborne particulate matter originating from feedyards to transfer bacteria, AMR determinants [2], and antibiotics [3] to surrounding environments; however, little work has been done to characterize the bacterial communities present within particulate matter. Moreover, information regarding bacterial population dynamics within the feedyard environment during the finishing period is limited. It is important to understand the dynamics of bacterial communities that persist in cattle and the environment to determine the ability of particulate matter to transmit AMR determinants to nearby environments and explore potential mitigation strategies. Our study aimed to characterize and compare the metagenomes of cattle feces and the feedyard environment as a function of tylosin and/or probiotic administration, and pen environment change and artificially model environmental manure pack desiccation to dust to characterize the metagenomic changes experienced in the cattle feedyard environment over time. We performed 16s rRNA amplicon sequencing on environmental and fecal samples from a 2x2x2 full factorial longitudinal study in which tylosin, an *Enterococcus faecium/Saccharomyces cerevisiae* probiotic, and pen change were investigated. Bioinformatic analyses of sequence data were performed using the QIIME2 pipeline and associated plug-ins to evaluate and compare microbiomes. Bacterial communities present within cattle feces, the environment, and airborne particulate matter exhibited discordant bacterial communities. Day and pen change were frequently associated with changes in bacterial populations within the same sample type. Treatment with tylosin and/or probiotics was not associated with alterations in the bacterial community structure within cattle feces, the feedyard environment or particulate matter during the finishing period. The environment likely plays a role in the persistence and transmission of certain bacterial taxa in the feedyard. Understanding bacterial community changes as a result of antibiotic and probiotic administration allows for a better understanding of the effects of these commonly administered therapies in cattle and the environment.

References

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O3

The effect of manure amendment on the soil grassland resistome

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Manure fertilisation is one of the routes through which animal fecal bacteria enter the environment and can act as source of resistance genes. In a field study in the Northern part of the Netherlands, the influence of manure fertilisation on the soil microbiome and resistome of grasslands was investigated, and linked to studies on horizontal gene transfer. As observed from analysis of resistance genes by qPCR, manuring initially increased levels of erm(B) and tet(W) by ~1 log copies/kg across soil textures, after which levels gradually declined. An increase was also seen in adjacent surface waters. The gene decay in soils was strongly dependent on the type of ARG. Recovery of ARG levels was predicted to occur after 29 – 42 days. Similarly, targeted metagenomics showed a substantial increase of ARGs in soils and adjacent waters, while no change in the overall bacterial community was found despite an increase in specific families. Targeted metagenomics also was able to demonstrate that not all resistance genes increase after manuring – some genes potentially hosted by soil bacteria rather declined in their relative abundance. Last, we studied whether horizontal gene transfer could occur under conditions representative of manure application, by use of fluorescently labelled strains. In model systems for manure-amended soils, HGT could indeed be detected. However, the time window for (observable) HGT events was limited by the survival of manure-borne donor bacteria. All in all, manure-incuded changes were observed in all different test systems used, however these changes were transient in nature.

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O4

Dynamics of cephalosporin-resistant in *Escherichia coli* in gulls highlight the importance of longitudinal wildlife studies

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Wildlife is generally overlooked as part of the environment but plays an important role in relation to antimicrobial resistance (AMR). The impact of migrating and vagrant species on the dissemination of the AMR remains unclear. To resolve this, it is crucial to understand whether wild animals are just temporary carriers of the AMR or if the resistant bacteria can be maintained in their gut. We performed a longitudinal experiment to analyse the dynamics of cephalosporin-resistant *Escherichia coli* in Caspian gulls (*Larus cachinnans*).

Five non-flying chicks were sampled at their nesting site and then transferred into captivity for 10 weeks where they were held together till the end of the experiment. The set of samples consisted of cloacal swabs obtained periodically every two weeks. Selective cultivation was conducted, from which five colonies were taken, and resistant *E. coli* isolates were tested for the presence of resistance genes and sequenced via whole-genome sequencing (Illumina, MinION) to obtain their genomic profiles and complete plasmid sequences.

The data showed significant changes in the presence of various sequence types (STs) over time and their transmission between individual birds. At the first sampling, most birds were colonized by *E. coli* ST11893 with *bla*$_{\text{CMY-2}}$ disrupting colicin-encoding gene on F34:A:-B:- plasmid. Only one gull carried CTX-M-1-producing *E. coli*. The *bla*$_{\text{CTX-M-1}}$ gene was carried by a multi-drug resistance colicin-associated IncI1/ST3 plasmid. In next samplings, the original predominating *E. coli* disappeared and IncI1/ST3 plasmid started to spread in various *E. coli* genotypes in all birds with a single ST predominating over time. The carriage of cephalosporin-resistant isolates decreased two months after the beginning of the experiment.

Our results point out that gulls can carry cephalosporin-resistant bacteria for over a month which leads to further spread of AMR. We observed that birds can exchange bacteria among each other which may result in the emergence of new and possibly more successful STs. Association of AMR plasmids with colicin production seems to be an important feature in their successful spread. Our data prove the importance of longitudinal studies, providing information on dynamic changes in the gut resistome over time.

O5

Spatial spreading of antibiotic resistance genes by gulls

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Due to their opportunistic behaviour (feeding on human waste in landfills or roosting in wastewater treatment plants), migratory gulls play a key ecological role in the dissemination of antibiotic resistance (AR) at the interface of human-domestic-wild environments. However, the factors determining AR spread by these birds are complex and not fully understood. We used the lesser black-backed gull (*Larus fuscus*, the second most abundant migratory waterbird in Andalusia, Spain) as a model and compared their faecal bacterial communities and their associated antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) between functional units (areas that gulls use for roosting and feeding consistently during their wintering) and within different habitats (wetlands *vs.* landfills). The richness and diversity of faecal bacterial communities were higher in faeces collected from more polluted sites. The gull faecal carriage of ARGs conferring resistance to aminoglycosides, chloramphenicols, and vancomycin varied spatially but were similar across wetlands and landfills, probably because of the high connectivity between these two types of habitats. The comparison between the abundance of ARGs and MGEs in gull faeces with that in the surrounding environment (soil and surface water) did not reveal the same pattern for all antibiotic families; ARGs conferring resistance to quinolones, vancomycin, and genes coding for integron-integrase gene, were higher on water and soil than in gull faeces, while for tetracyclines, cloramphenicoles, macrolides and genes encoding for other MGEs (insertion sequences) were the opposite. Our results demonstrate that the faecal microbiota of birds and their associated ARGs changed spatially and that birds using landfills for feeding and
roosting are more prone to disseminate antibiotic resistance determinants and potential pathogens across different biomes.

06

Compartmentalization of antibiotic resistance between phages and bacteria in a river - wastewater treatment plant continuum

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Transduction is probably one of the major horizontal transfer mechanisms allowing antibiotic resistance to spread between commensal, pathogenic and environmental bacteria [1]. If this mechanism is well described *in vitro*, its frequency is still difficult to evaluate in the environment and the parameters and actors that govern it remain poorly known [2,3]. In this work, we studied the compartmentalization of few antibiotic resistance genes and mobile genetic elements in bacteria and phages of microbial communities present in the water of the Meurthe River, and in the effluents of a wastewater treatment plant discharging into it over time. Bacterial and phage DNAs were extracted separately, carefully checking for the latter that no free/non-encapsidated DNA had contaminated the extracts. The 16S rDNAs and a panel of 10 antibiotic resistance genes and 9 mobile genetic elements selected for their environmental and/or clinical relevance were then quantified by quantitative or digital PCR in the extracts. Quantification of 16S rDNA in DNA purified from phage fractions confirmed the presence of transducing phages containing chromosomal DNA in all the different microbial communities. While in river water, transducing phages are not very abundant and only carry a maximum of 0.08% of the 16S rDNA present in a whole microbial community, this value can reach 16.3% in wastewater treatment plant effluents, thus demonstrating the very high proportion of transducing phages released in the environment through this medium. This trend was also observed with the different antibiotic resistance markers monitored where, for example, a maximum of 0.01% of class 1 integrons were carried by phages from river microbial communities while up to 2% of them were carried by phages from effluent microbial communities. In extreme cases, effluents even had more than half of the *sul1* genes and more than a quarter of the *vanA* genes they contained that were vehiculated in the phage fraction! Overall, the tendency of antibiotic resistance genes and mobile elements to be encapsidated was variable from marker to marker and from medium to medium. While the effluents were usually much concentrated in antibiotic resistance determinants than the river water, no significant increase in antibiotic resistance could be detected in the river water downstream the treatment plant, likely because of the dilution effect. These results, however, arises the question of the impact of bacteria and especially that of the numerous transducing phages released in the river through the effluents with respect to their respective roles in disseminating antibiotic resistance in the environment.

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O7

Occurrence of 41 sanitary indicators in digestates derived from different anaerobic digestion processes and various raw organic wastes

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To date, there is no scientific consensus on the fate of pathogenic bacteria present in raw organic wastes and the sanitary quality of digestates. This work aims to characterize the influence of the nature of raw wastes as well as the types of processes on the sanitary quality of digestates. For this purpose, the occurrence and the fate of 41 sanitary indicators including pathogenic bacterial species, antimicrobial resistance genes as well as new indicators of health hazard such as virulence factors carried by mobile genetic elements were monitored during anaerobic digestion (AD). Three full-scale plants were selected to study the effect of the key parameters of the AD process (type of alimentation, number of steps, temperature, pH). While the impact of the nature of raw wastes has been studied with 3 lab-scale reactors where the AD process was similar (semicontinuous, mesophilic) but the proportions of inputs have been varied (mainly fecal matters supplemented or not with straw or straw and zeolite). First, the prevalence of these indicators was measured in digestates and compared with that measured in raw wastes. Of the 13 species studied, only one (*Streptococcus agalactiae*) was completely removed, regardless of the process and nature of the waste used. For virulence factors and mobile genetic elements, no significant decrease in prevalence could be detected. In a second step, absolute quantification of the indicators displayed a high prevalence in raw wastes has been done using a Digital Droplet PCR system. Regarding the effect of the nature of raw waste, the reactors fed with fecal matter supplemented with straw (Lab2) or with straw and zeolite (Lab3) have a higher hygienization efficiency compared to reactor fed uniquely with fecal matter (Lab1). Thus, 2 pathogenic species (*Enterococcus faecalis*, *Enterococcus faecium*) and 3 mobile genetic elements (*intI1*, *intI2* and IS*26*) were significantly decreased in both reactors (Lab2 and Lab3). Nevertheless, 1 mobile genetic element (*intI1*) and 1 antibiotic resistance gene (*tetO*) have been significantly increased in Lab1. Interestingly, of 13 polycyclic aromatic hydrocarbons (PAHs) and 13 pharmaceutical products measured in this study, the quantities of 11 PAHs and 11 pharmaceutical products, were significantly higher in Lab1 samples (treated or not) than in Lab2 and Lab3 samples. The high concentration of some micropollutants such as triclosan found in Lab1 samples could explain the lower hygienization efficiency of AD process in this reactor compared to the other reactors (Lab2 and Lab3). Regarding the impact of the type of process, the fed-batch reactor had a better hygienization effect with a total reduction of a virulence factor (*ybtA*), involved in the production of siderophores, and a significant reduction of enterotoxigenic *Escherichia coli*. This study showed that the sanitary quality of digestate is first related to the sanitary quality of raw waste and then to the employed process.

08

Microplastics increase the selective potential of antibiotics at sub-inhibitory concentrations

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Microplastics (MPs) are major pollutants that are massively released into the environment. In urban waters, biofilms can form on plastic surfaces and thus MP particles might affect environmental bacteria and their associated resistome. In addition, antibiotics may adsorb onto plastic surfaces and have a greater effect on plastic-associated bacteria than on planktonic bacteria. Therefore, the presence of MPs in the environment could pose a risk for antibiotic resistance development and dissemination in environmental settings. The goal of this study was to determine the impact of microplastics in combination with antibiotics on environmental bacterial growth and antibiotic resistance. We hypothesized that the presence of MPs could increase the selective potential of antibiotics at sub-inhibitory concentrations on environmental bacteria and their associated antibiotic resistance genes (ARGs). DNA was extracted from urban river bacteria enriched in TSB medium or sterile water over 72 hours, with and without microplastics and gentamicin and ciprofloxacin at sub-inhibitory concentrations. The 16S rRNA and gentamicin resistance genes were amplified by qPCR to determine pollutant effects on growth and ARG selection. In addition, the influence of MPs and antibiotics on river water communities was evaluated by sequencing the 16S rRNA gene. Bacterial abundance was lower in the MP fraction than in the liquid fraction (both with or without MPs). Antibiotics had no effect on bacterial abundance in any fraction and where thus overall sub-inhibitory. Bacterial exposure to both gentamicin and ciprofloxacin at sub-inhibitory concentrations induced a larger shift in baterial community composition in the MP fraction than in the liquid fraction (with or without MPs), with an increase in the relative abundance of *Citrobacter*, *Klebsiella* and *Pseudomonas*. Gentamicin and ciprofloxacin selected for gentamicin resistance genes in the MP fraction, both in TSB medium and in sterile water. Therefore, our results are consistent with antibiotics at sub-inhibitory concentrations having a larger impact on environmental antibiotic resistance in the presence of MPs. This study adds to the concerns related to the role of microplastic pollution on the emergence of antibiotic resistance in the environment.

O9

Do microplastics offer a suitable surface for antibiotic resistance spread into the open environment?

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Plastic pollution represent an anthropogenic stressor with presumably large ecological implications. In particular, microplastics (<5 mm particles) collectively constitute an abundant artificial surface for biofilm formation, where Horizontal Gene Transfer (HGT) of Antibiotic Resistance Genes (ARGs) could be enhanced. However, still little is known about the contributions of microplastics to microbial community change and ARGs spread. In this context, we propose a multidisciplinary study to address these potential contributions, in relation to agricultural practices, natural environments, food webs and human health. We set-up microcosms with microplastics (seven most common plastics; 10 % of the samples' volume), antibiotics (ciprofloxacin, vancomycin, cefotaxime - 1100 mg/L) and three environmental matrices (irrigation water and sediments; compost; manure). Shotgun metagenomics and microbiome data analysis revealed the effects of sample type, microplastic and antibiotic additions on phylogenetic community compositions and on the metabolic potential of assembled genomes(Fig.1). Resistome analyses accounting for chromosomic or plasmidic genomic location allowed us suggest for ARGs spread mechanisms within environments. An overrepresentation of antimicrobial gene copies was found, especially for streptomycin, tetracycline, vancomycin, cefotaxime and ciprofloxacin. The microplastic additions affected copy numbers of a few antibiotic resistance genes such as those encoding resistance to e.g., streptomycin and tetracycline. Antibiotics addition was associated with differentially abundant genera such as *Achromobacter*. To conclude, interaction effects between microplastics and antibiotics may promote antibiotic resistance spread in a wide range of natural and anthropogenic environments.

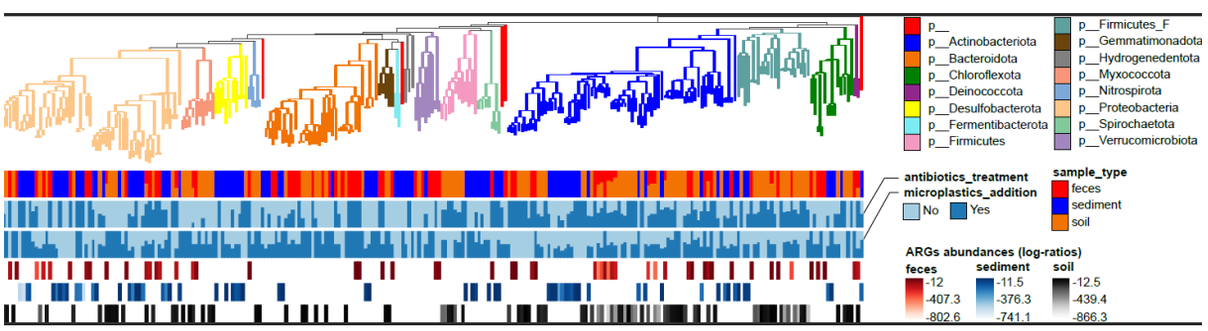

Fig.1. Phylogenetic tree with barplots showing the sample type, treatments and the log ratio of ARGs abundance.

O10

Can Antimicrobial Resistance Genes Travel Across the Atlantic Ocean?

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The presence of antimicrobial resistance genes (ARGs) in the air has been the topic of a flourishing literature in the past years. Recently, they have been detected in clouds at mid-altitude, attesting to their potential transport over long distances (Rossi *et al*., 2023). In this work, marine sources were highlighted as significant contributors of ARGs, especially regarding quinolone resistance. So far, however, the emissions of ARGs from seas and oceans remain under-studied, in particular because of the numerous sampling constraints.

In this study, the presence of ARGs over the Atlantic Ocean's surface was specifically addressed through a 20-days transatlantic campaign from Brest, France, to Woods Hole, on the west coast of the USA, aboard a sailing ship specially equipped with a microbiology lab. Every day and night, aerosols were collected for periods of 1 hour using a high-throughput point source sampler (SASS 3100), at a rate of ~300 L air/min. Additional sampling apparatuses (filter-holders connected to individual pumps) were deployed to collect samples over periods of 24h, at a flow rate of ~7 L/min, in triplicate. For each air samples, a panel of 33 different subtypes of ARGs (including quinolones, β-lactams, macrolides, tetracyclines, sulfonamides, aminoglycosides, vancomycins and one mobile genetic element) was monitored by qPCR and related to bacterial diversity (MiSeq Illumina) and concentration (qPCR 16S). These were further related to the backward trajectories of the corresponding air masses reaching the sampling site, using the ECMWF ERA-5 CAT 3D kinematic trajectory model.

Preliminary results highlight a high variability in the concentration of airborne bacteria at the ocean surface, averaging ~10^3 cells.m^{-3} of air based on 16S gene abundance; this tended to increase as the boat moved away from the coasts. Regarding antibiotic resistance, 22 different subtypes were recurrently detected, dominated by quinolone, macrolide and tetracycline resistance genes. Concentrations were highly fluctuating, ranging from few copies to ~10^4 copies per m^3 of air, and ARGs' profiles differed between day/night sampling, with values that tended to be higher during the night. The data are currently being analyzed with respect to air masses geographical origins and bacterial diversity. This work highlights that ocean surfaces could contribute significantly to ARGs inputs in the atmosphere and to their global spread.

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Session 2 - Monitoring and molecular epidemiology of antimicrobial resistance

O11

Use of genomics to characterise antimicrobial resistances present within livestock on UK farms

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The rise in antimicrobial resistance (AMR) in bacteria due to overuse or continued misuse of antibiotics in agriculture, human and veterinary medicine is a global threat. The food chain and the environment are also affected, resulting in widespread dissemination of multi-drug (MDR) bacteria, which is affecting our ability to treat bacterial infections. The global burden of AMR has continued to increase, and it has been estimated that there are 75,000 human fatalities a year due to MDR bacteria, and an increased morbidity that is affecting patient outcomes. To tackle the risk of AMR the United Kingdom has developed a National Action Plan which aims to evaluate and control it in the UK. The Animal and Plant Health Agency (APHA) has been central in its implementation, conducting research and surveillance within UK livestock, working in a One-Health context, as humans, animals and the environment are inherently linked. To survey and gain insight into the routes and mechanisms of transmission of AMR within the livestock compartment we have used genomics, so new and emerging AMR threats can be identified promptly, the flow of AMR genes can be tracked accurately and the risk to human health assessed in a One-Health context. Such work is helping to protect public and animal health through better control of AMR in bacteria occurring in livestock, their environment, and the food chain.

O12

The role of FDA's Veterinary Laboratory Investigation and Response Network in monitoring antimicrobial resistance of animal pathogens

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Background: The Veterinary Laboratory Investigation and Response Network (Vet-LIRN) is an FDA program created in 2010 to coordinate a network of veterinary diagnostic laboratories. In 2017, Vet-LIRN initiated an Antimicrobial Resistance (AMR) Monitoring Program focused on monitoring AMR profiles in veterinary and zoonotic pathogens isolated from animals at veterinary clinics and diagnostic laboratories in the U.S. and Canada. Vet-LIRN's AMR program supports the national initiative to Combat Antibiotic Resistant Bacteria (CARB) and FDA's Center for Veterinary Medicine's multi-pronged, multi-year strategy designed to slow the emergence of resistance arising from the use of antibiotics in animals. It supplements existing efforts by the National Antimicrobial Resistance Monitoring System (NARMS), which monitors AMR of foodborne pathogens in humans, retail meats, and food animals at slaughter.
Methods: Vet-LIRN laboratories provided antimicrobial susceptibility testing (AST) data from clinically relevant bacterial isolates from animals: *Salmonella enterica* from any animal host, and *Escherichia coli* and *Staphylococcus pseudintermedius* isolates from dogs, using a commercially available testing platform. Laboratories followed Clinical and Laboratory Standards Institute AST testing methods. Laboratories sequenced a subset of isolates and submitted the whole genome sequencing (WGS) data to the National Center for Biotechnology Information (NCBI) submission portal, where they were assembled and analyzed using NCBI's bioinformatics pipelines. All sequencing data are publicly available. Vet-LIRN has partnered with NARMS to include animal pathogen AMR data into the NARMS integrated report and create publicly available data dashboards.
Results: Since 2017, the Vet-LIRN AMR program has provided a wealth of information to detect emerging antimicrobial resistance, understand the effectiveness of FDA-regulated products over time, and foster antimicrobial stewardship in veterinary settings. For example, in 2019 the carbapenem resistance gene, blaNDM-5, was identified for the first time in an animal pathogen in the U. S., in a 2018 isolate of *E. coli* from a dog in Philadelphia. Over 15,000 isolates have undergone antimicrobial susceptibility testing, and more than 4,000 isolates have been sequenced with data publicly available under NCBI BioProject PRJNA314609. Vet-LIRN AMR data dashboards include resistance mechanisms from genomics data, along with the percent resistance and MIC distributions for each of the antibiotics included in the panels.
Conclusions: By making AMR monitoring data publicly accessible, the Vet-LIRN AMR monitoring program facilitates international One Health research and monitoring.

O13

Genomic diversity of porcine LA-MRSA CC398 isolates collected in the German national resistance monitoring program GE*RM*-Vet between 2007 and 2019

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Background and objectives: The livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) clonal complex (CC) 398 lineage is known for its frequent multiresistance to antimicrobial agents and a low host specificity [1]. Colonized pigs represent a risk of LA-MRSA CC398 colonization or infection for occupationally exposed people, through which such isolates might be spread further within the human community [1]. Here, we investigated a collection of unrelated LA-MRSA CC398 isolates for evolutionary relationships and genomic diversity with focus on their virulence and antimicrobial resistance (AMR) properties.
Materials and methods: In total, 178 unrelated LA-MRSA CC398 were collected in the German national resistance monitoring program GE*RM*-Vet from diseased swine between 2007 and 2019. Whole-genome sequencing was carried out, followed by molecular typing and sequence analysis. A minimum spanning tree based on core-genome multilocus sequence typing was constructed and antimicrobial susceptibility testing was performed according to CLSI standards [2].
Results: The LA-MRSA CC398 isolates showed close phylogenetic relationships and a wide molecular variety, including 13 *spa* types as well as 19 known and four novel *dru* types. Several toxin-encoding genes, including *eta*, *seb*, *sek*, *sep* and *seq*, were detected. The isolates harbored a wide range of AMR properties mirroring the proportions of the classes of antimicrobial agents applied in veterinary medicine in Germany. Several novel or rare AMR genes were identified, including the novel macrolide-lincosamide-streptogramin B resistance gene *erm*(54), the lincosamide-pleuromutilin-streptogramin A resistance gene *vga*(C), and the phenicol-lincosamide-oxazolidinone-pleuromutilin-streptogramin A resistance gene *cfr*. Many AMR genes were part of small transposons or plasmids. Clonal and geographical correlations of molecular characteristics, AMR and virulence genes were more frequently observed than temporal relations.
Conclusion: This study provided insight into diversity and population dynamics of the epidemic porcine LA-MRSA lineage in Germany over a 13-year-period. In order to detect new emerging, possibly more dangerous clones and to prevent LA-MRSA transmission between livestock farms and entries into the human community, a large-scale LA-MRSA monitoring is essential.

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O14

Antimicrobial resistance in pathogenic bacteria of Victorian cattle

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Australia has one of the most conservative approaches to the use of antimicrobials in food producing animals in the world. Registration of antibiotics for use in animals is tightly controlled, prescribed by veterinarians and the use of fluoroquinolones, colistin and fourth generation cephalosporins are prohibited for use in food producing animals to minimise their impact on human medicine. In Australia, surveys have been undertaken for antimicrobial resistance (AMR) in commensal bacteria from live cattle at slaughter, however little knowledge is shared on the AMR status of pathogenic bacteria isolated from cattle in Australia. Understanding the presence of AMR and particularly multi-drug resistant (MDR) pathogens on farm is a critical step in understanding the risks of AMR/MDR to the farm and the broader industry, to support biosecurity and public health.

This project focused on 11 pathogenic bacterial isolates (10 *Salmonella* spp and 1 *E. coli*) taken from significant disease investigations of cattle in Victoria, submitted to the Agriculture Victoria Research (AVR) Veterinary Diagnostics Laboratory (VDL) over a 3-year period (Feb 2020 - Feb 2023). The isolates were selected based on their phenotypic resistance to 2 to 5 antibiotics, sequenced using the Illumina NovaSeq and bioinformatic analysis conducted. Antimicrobial resistance genes (ARGs), mobile genetic elements and heavy metal resistance genes were identified on the genomes of all 11 isolates. Five isolates that carried ARGs for different antibiotic classes were selected and further sequenced on MinION to complete the genome and investigate the genetic links between the genes and the mobile genetic elements. The presence of ARGs identified in these isolates may not be directly related to the particular antibiotics used on farm and may be the result of co-selection of other antibiotics or heavy metals.

O15

Clonal and plasmidic dissemination of critical antimicrobial resistance genes in dairy cattle in Québec, Canada.

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Tackling AMR should be a priority for all public health stakeholders. Increasing the judicious usage of antimicrobials (AM) in food production has proven efficient to limit AMR. However, its effect on resistance gene dissemination in animal populations is not known. The province of Québec (Canada) enacted a new legislation in February 2019, to limit usage of critical AM in production animals.

The aim of our study was to investigate the putative link between ESBL/AmpC *E. coli* isolated pre- and post-legislation and to determine the presence of plasmid carrying genes responsible for resistance to crucial AM in these isolates.

We collected fecal samples from calves, cows, and the manure pit in 87 dairy farms in Quebec approximately 2 years before and two years after the enactment of the legislation. The whole genomes of 183 (91 before and 93 after) putative ESBL/AmpC *E. coli,* isolated after cefotaxime enrichment, were sequenced. Their phylogenetic characteristics (MLST, serogroup, cgMLST) and the presence of virulence and resistance genes and replicons were examined. A maximum likelihood phylogenetic tree was constructed based on SNP data.

We identified 10 clonal lineages (based on cgMLST) and 6 clones (SNPs < 35). Isolates belonging to these clones could be found on different farms pre- and post- legislation, strongly suggesting clonal dissemination of antimicrobial resistance genes in the population during this 4-year period (cf Figure1). All clones were MDR, Clone 2 being noteworthy, due to the presence of resistance genes for macrolides, fluoroquinolones and third generation cephalosporines and of virulence genes *afa* and *papC*. Our data also strongly suggest the presence of at least two epidemic plasmids belonging to the incompatibility groups IncHI2 and IncY and carrying *qnrs1* and $bla_{CTX\text{-}M}$, and one IncC plasmid carrying $bla_{CMY\text{-}2}$ gene. These different plasmids were identified pre- and post- legislation, suggesting that they are persistant despite the changes in antimicrobial usage (data not shown).

Clonal and plasmidic dissemination of resistance genes persist in the dairy population in Quebec despite limiting the use of critical antimicrobials. Further monitoring is essential to better understand the emergence and persistance mecanisms of these genes. In particular, it will be important to determine the capacity of Clone 2 to become a high risk clone.

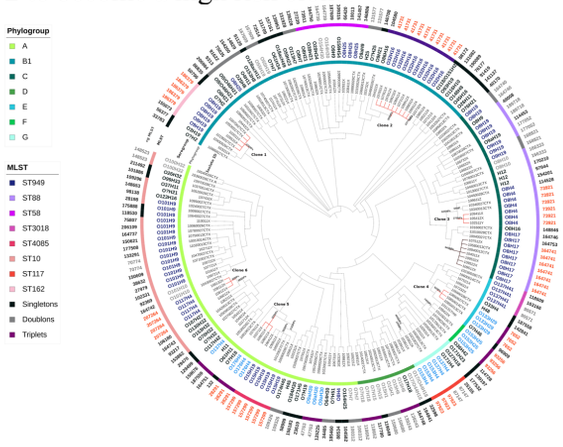

Fig. 1. Phylogenetic tree of 183 ESBL/AmpC *E. coli* isolates based on the SNP differences. Isolates with an ID ending in CTX belongs to the "pre-legislation" period all other isolates belong to the post-legislation period. The two digits in the 3rd and 4th position of the ID represent the farm number.

O16

A phylogeny study of ß-lactam-resistant *E. coli* from Belgian calves

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In a One Health context, some antimicrobials classified by WHO as "critically important for human medicine" have a restrictive use in veterinary medicine in the European Union, among which 3rd and 4th generation cephalosporins (3GC and 4GC), from ß-lactam, a family widely used in human and veterinary medicine. To evaluate the impact of such restrictions, we have characterized the epidemiology of ß-lactam resistance in *Escherichia coli* isolated from diseased calves in Belgium. During three calving seasons (2017–2020) *E. coli* were collected from young diarrheic or septicemic calves and their phenotypic resistance profiles were determined, showing a constant high rate of ß-lactam resistance, but a decrease of 3GC/4GC resistance [1]. To characterize these observations at the genomic level, we sequenced 642 *E. coli* from our collection, including 522 3GC/4GC resistant isolates. Their evolution during the three seasons and their relationships with other animal and human *E. coli* were studied.

The 642 calf *E. coli* showed a broad diversity of ß-lactamase patterns, but are consistently multidrug resistant. Phylogenetic analysis shows the striking importance of phylogroup C, with 30% of ST88, and none isolate belongs to the B2 phylogroup, associated with human extraintestinal infections. The distribution is similar during the three seasons. Most of the *E. coli* (78%) belong to 13 different STs, which were analyzed jointly with genome sequences from the same STs downloaded from Enterobase. We identified some lineages showing a local dissemination (Figure 1). On the other hand, the intermixed human and animal isolates in some STs reveal its zoonotic potential.

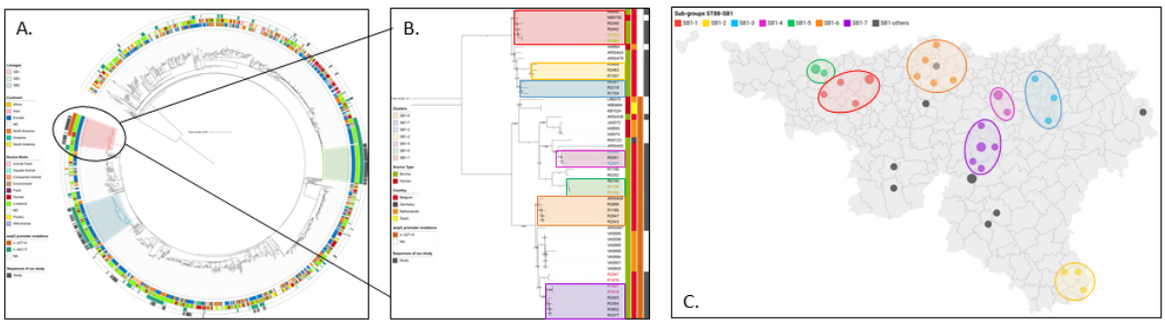

Fig. 1. ST88 phylogeny (A), with three major lineages identified (red, blue and green). Focus on the ST88-red lineage (B) and the geographical repartition of isolates in this lineage (C). The colors in B and C are consistent.

In conclusion, the reduction of C3G resistance was not associated with changes in resistance mechanisms and population structure. In the 13 dominant STs, isolates clustering reflected either local transmissions or/and endemicity. For some of them, a zoonotic potential was observed. This is to our knowledge the first large scale study in diseased calves, as most studies in livestock are focusing on carriage isolates, possibly explaining the specificity of our findings.

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O17

Multidrug-resistant *Escherichia coli* isolated from slaughtered broilers in Ilorin, Nigeria

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The occurrence of multidrug-resistant *Escherichia coli* in food-producing animals including poultry poses economic losses and public health hazards. Genomic information on the molecular diversity of E. coli will aid public health decision-making, increase food safety, improve understanding of the molecular basis of antimicrobial resistance (AMR), and could provide vital data for the improvement of *E. coli* vaccines. Hence, we conducted genomic characterization of *E. coli* isolated from broilers in Ilorin, Kwara State, Nigeria. A structured questionnaire was interviewer-administered to 125 poultry farmers in Ilorin to determine their knowledge, attitude, and perception towards prudent antibiotic use and its implications. Also, broiler faecal samples (n=1000) were screened for *E. coli*. Phenotypic antibiotic susceptibility testing was done using both the disc diffusion assay and molecular characterization was done using whole genome sequencing. Many poultry farmers (63.0%) used unprescribed antibiotics, there was zero compliance with antibiotic withdrawal periods, and 69.6% of them were aware of AMR. Older farmers, male respondents, those with tertiary education, and those with larger flock sizes were more likely to have satisfactory knowledge of AMR. *E. coli* was isolated from 403 samples. A total of 189 samples were multidrug resistant. WGS of the 31 ESBL-producing multi-antibiotic resistant isolates revealed that revealed high genomic diversity. The resistome revealed that the isolates harboured resistance determinants to fluoroquinolone (n=8), aminoglycoside (n=3), tetracycline (n=3), beta-lactam antibiotics (n=11), chloramphenicol (n=4), foliate pathway antagonist (n=4), fosfomycin (n=3), macrolides (n=3), and two disinfectant resistance genes which conferred resistance to hydrogen peroxide and quaternary ammonium compounds. Mutations in the *gyrA* gene conferring resistance to fluoroquinolones were also detected. There was a positive correlation between phenotypic resistance patterns and the antibiotic resistance genes (ARGs) that were detected in the sequenced isolates. Isolates were of diverse sequence types (ST) with 12.9% belonging to ST117 and the virulome comprised of diverse virulence factors such as adhesins, iron acquisition genes, toxins, and protectins. The mobilome consisted of several Col-plasmids (Col440I, ColRNAI, and ColpVC) and the predominant IncF plasmids (IncFIB, IncFII) belonged to the IncF64:A-:B27 sequence type. The isolates were of diverse serotypes with O17:H18 being the most prevalent (9.7%) and were classified into five phylotypes (A, B1, B2, E, and F) with phylotype F being the most prevalent (32.3%). Intact phages belonging to the *Myoviridae* (77.4%), *Siphoviridae* (51.6%), *Inoviridae* (29%), and *Podoviridae* (6.5%) viral families were detected. No antibiotic RGs were identified on any of the phages. The isolates were clustered into five different clades and 71% of them had >80% similarity to human pathogens. The occurrence of multi-antibiotic-resistant *E. coli*, co-occurrence of antibiotic- and disinfectantresistant genes, and ESBL-*E. coli* isolates that were potential human pathogens in poultry were established in Kwara State. Our findings suggest that poultry are potential carriers of clonally diverse and pathogenic pathogens that pose a public health threat. Hence, critical control points in the poultry value chain are urgently needed.

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O18

Fluoroquinolone resistance in *E. coli* in broilers: mapping the spread

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Objective
Although restrictions on the use of fluoroquinolones in livestock have been imposed in Belgium since 2016, high levels of fluoroquinolone (FQ) resistance are still found in commensal *E. coli* in broilers. The purpose of this study was to describe the FQ resistance prevalence in *E. coli* and to investigate the role of day-old chicks and the environment in the dynamics of the spread within different flocks.
Materials and methods
On 29 broiler farms, antibiotic use was monitored and both water and environmental samples were collected prior to arrival of the chicks. Thirty broilers were sampled per farm on days 0 (before entering the stable), 3 and 35 of the production round. In all samples, total *E. coli* and FQ resistant *E. coli* isolates were quantified by plating on MacConkey agar without and with 0.25 µg/ml enrofloxacin (ECOFF). All isolates were identified using MALDI-TOF MS. A selection of the isolates was submitted for whole genome sequencing to investigate their phylogenetic relatedness using core-genome MLST (cgMLST) analysis, and to detect resistance determinants.
Results
Before they entered the stable, the day-old chicks carried FQ resistant *E. coli* in 82.8% of the farms and FQ resistant *E. coli* were found in the environment on boots (10.3%), hygiene locks (24.1%), drinking cups (10,3%), feeding pans (20.7%), and stable floors (24.1%). According to cgMLST, overall there was a large diversity of FQ restistant *E. coli* strains. However, identical FQ resistant isolates were found on day 0 and day 35, suggesting that FQ resistant isolates present in the environment at the start of a production round or in day-old chicks, remained present until slaughter, even though no FQs were used.
Conclusion
The continued presence of FQ resistant *E. coli* in Belgian broiler farms is likely the result of both a historical contamination at the farm level and a continuous influx along the production chain.

O19

In-vitro validation of AMR genes prevalence revealed by WGS in *Campylobacter jejuni* isolated from chicken livers in Spain.

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Campylobacter is the most common cause of foodborne human gastroenteritis worldwide. The largest contributors of human campylobacteriosis in developed countries are poultry and poultry products. There have been outbreaks of campylobacteriosis attributed to undercooked chicken livers. In previous studies, we determined the prevalence of *Campylobacter* in chicken livers, highlighting their potential relevance as a source of human campylobacteriosis. On the other hand, the high prevalence of antimicrobial resistant (AMR) strains in *Campylobacter* spp. from poultry has become a growing concern since these strains can be transferred to humans causing an antibiotic-resistant infection. The aim of this study was to assess the AMR prevalence of a selection of *C. jejuni* isolates from chicken livers and the characterization of the AMR gene presence through whole-genome sequencing (WGS) and comparison with Resfinder database. A total of 33 liver isolates obtained from carcasses at slaughter from healthy chicken from 21 different farms from Catalonia (north-eastern Spain) were tested, obtaining valid results from 24 isolates since the remaining nine were excluded due to poor growth. AMR to at least one antimicrobial was revealed by the broth microdilution method with EUCAMP3 plates, in 91,67% of the isolates. The most common resistances found were to ciprofloxacin (79,17%) and tetracycline (66,7%), and the AMR profile ciprofloxacin-tetracycline was the most frequent (50%). One isolate was multidrug resistant (profile ciprofloxacin-tetracycline-ertapenem) while two isolates were pansusceptible. DNA was extracted from all the isolates and submitted to shot-gun sequencing (Illumina HiSeq). The raw sequences were processed to be quality filtered and the genomes were *de-novo* assembled (with SPADES) using PATRIC. The contigs obtained were inspected for AMR gene presence using the ResFinder 4.1 tool (https://cge.cbs.dtu.dk/services/ResFinder). All the isolates showing ciprofloxacin resistance *in-vitro* carried the point mutation in the gyrase gene (T86I) which confers resistance to quinolones. For the 16 tetracycline-resistant isolates, all but one carried the Tet(O) gene. All isolates carried the genes coding for the CmeABC multidrug efflux pump which has been shown to contribute to both intrinsic and acquired resistance to tetracycline and might explain the tetracycline-resistance profile of the remaining isolate. The isolate showing ertapenem resistance was the only carrying the blaOXA-185 gene, while all isolates carried the blaOXA-61 gene. These genes are associated with b-lactam resistances, however, since other carbapenems apart of ertapenem are not included in the EUCAMP3 plates, it could not be confirmed *in vitro*. Additionally, the information provided by WGS allowed to predict a variety of AMR profiles that included resistances to antibiotics not included in the EUCAMP3 plates, particularly to ampicillin and nalidixic acid. Altogether, these valuable results show the risk posed by chicken livers in the transmission of AMR *C. jejuni* and the power of WGS in providing additional meaningful information on the AMR potential of those isolates.

O20

Antimicrobial resistance determinants in bacteria isolated from retail chicken and pork meat across New South Wales, Australia

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Introduction and aims. Few studies have been conducted in Australia to determine the diversity of antimicrobial resistance in bacteria isolated from retail meat. The aim of this study was to utilize whole genome sequencing to determine the diversity of resistance genes in bacteria isolated from retail meat samples across the most populous state of Australia; New South Wales (NSW).
Methods. A total of 404 meat samples (244 chicken and 160 pork) were purchased from 55 supermarkets across NSW, Australia. All samples were streaked onto Brilliance™ CRE/VRE/ESBL (CRE/VRE/ESBL), CAMPY (CAM), XLD (XLD), and MacConkey (MAC) agar. Whole genome sequencing was performed for 288 isolates, with all isolates that grew on CRE, VRE, ESBL, CAM, and XLD agar being prioritized, the remainder were isolates that grew on MAC agar. DNA libraries were prepared using plexWell™ 96 Kits (seqWell™) and sequencing performed on an Illumina NovaSeq™ platform (Illumina, Inc.) in a 150 bp paired-end format. Sequences were assembled using SPAdes, then annotated using RASTtk. Antibiotic resistance genes, plasmids, and virulence genes were identified using the Mobile Genetic Element finder tool from the Center for Genomic Epidemiology (CGE). The CGE's PathogenFinder 1.1 tool was used to predict the likelihood of isolates being pathogenic to humans. Antibiotic susceptibility testing was performed using Gram-negative and CAMPY Sensititre® plates containing critically- or highly-important antibiotics (CIA/HIA), as defined by WHO[1]. All plates were automatically inoculated and automatically read using Sensititre™ SWIN™ software.
Results. The 288 genomes were found to belong to 17 different genera and we detected an average of 1.53 antimicrobial resistance genes per genome (range: 0-11). Three isolates; one Serratia strain, one Aeromonas strain, and one Achromobacter strain, harbored a mobilizable colistin resistance (*mcr*) gene, identified as variants *mcr*-9, *mcr*-3.15, and *mcr*-5.1, respectively. A number of plasmid-associated quinolone resistance genes were also detected. Based on gene content, many of the isolates met the criteria for pathogenic pathovars of *E. coli*, including EPEC, ExPEC, and UPEC. Of the *E. coli* isolates, 98% were predicted to be pathogenic to humans, while 85% of the *Campylobacter* isolates were. Antibiotic susceptibility testing revealed that 12% (35/288) of isolates were deemed to be multiple drug resistant, of which 19% were cultured on CRE agar. Resistance to ciprofloxacin was observed for 14/288 (5%) of the isolates.
Conclusions. A vast diversity of bacterial species, antimicrobial resistance and virulence genes can be detected in bacteria isolated from retail chicken and pork meat in NSW. The identification of gene variants conferring resistance to CIA or HIAs not previously detected in Australian meat samples highlights the need for regular surveillance and monitoring of antimicrobial resistance.

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O21

Prevalence of Extended-Spectrum Beta-Lactam (ESBL) and Colistin Resistance in *Escherichia coli* Isolated from Broiler Chickens in Dressing Plants in the Philippines

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Antimicrobial resistance (AMR) in *Escherichia coli* from animals is a public health concern because of potential for transfer of antibiotic resistant bacteria and resistance genes to humans/ bacteria in humans via the food chain. Moreover, *E. coli* is commonly used as a sentinel indicator organism for monitoring AMR in enteric bacteria as it is found frequently in a wide range of hosts; may be exposed to antimicrobials in the intestine; acquires resistance easily; and provides potential indication of transferable resistance genes present in other intestinal Enterobacterales. The WHO categorises 3rd and 4th generation cephalosporins and colistin as critically important antimicrobials (CIAs) to human health. The European Medicines Agency (EMA) has also categorised resistance to these antibiotics as higher risk to public health (Category 2). A recent study on the antimicrobials used in backyard and commercial poultry farms in the Philippines, indicated that CIAs are frequently used in poultry farms [1]. However, published data on AMR of commensal *E. coli* from chickens raised for food are scarce; with only one publication indicating a resistance of 38.33% to nalidixic acid and 25% to ciprofloxacin but published data on resistance to cephalosporins and colistin are lacking [2].
In the present study, resistance to 3rd generation cephalosporins (ceftazidime & cefotaxime) and colistin and the prevalence and types of genes conferring resistance to these compounds were determined from 101 *E. coli* isolated from apparently healthy broiler chickens at slaughter. These isolates were obtained from chicken caecal content from National Meat Inspection Service (NMIS) accredited poultry dressing plants representing several regions of the Philippines. *E. coli* isolation was performed using standard microbiological methods and identification was carried out using VITEK 2 System (BioMerieux) and MALDI-TOF spectrometer (Bruker). Broth microdilution using the Sensititre™ System (Thermo Fisher Scientific) was used to determine the minimum inhibitory concentration (MIC) of 15 antibiotics representing nine antibiotic classes. MIC values were interpreted using EUCAST ECOFFs and whole-genome sequencing was carried out to determine the presence of AMR genes using NCBI AMR Finder Plus & APHA SeqFinder tools. Cephalosporin resistance was identified in 23/101 (23%) isolates. This resistance was due to the presence of *bla*DHA-1, *bla*CMY-2, *bla*CTX-M-55, *bla*CTX-M-15, or *bla*CTX-M-123 genes with *bla*DHA-1 the most common of these genes, 12/23 (52%). In addition, 10/101 (10%) isolates were resistant to colistin and harboured the *mcr*-1 resistance gene, six of which were also resistant to cephalosporins harbouring both the *bla*DHA-1 and *mcr*-1 genes. This study supports previous findings that apparently healthy farm animals can be reservoirs of ESBL and colistin resistant *E. coli*. This is of public health significance as the genes that encode for critically important antimicrobial resistance can spread to other bacterial species by horizontal gene transfer and can be transmitted to humans via food chain.

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O22

Risk factors for antimicrobial resistant *Aeromonas* spp. and *Escherichia coli* in the rainbow trout farm environment in Brittany, NW France

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Fish farms typically operate with surface water, which can be polluted with fecal bacteria from humans and livestock. As antimicrobial resistance (AMR) becomes widespread in humans, animals, and the environment, bacterial pollution of water used in food-producing environments is a rising concern. *Escherichia coli* is an indicator of faecal contamination in surface water and an indicator of AMR from the environment, thus the prevalence of antimicrobial resistant- *E. coli* is an interesting tool to monitor pollution in surface water, and in freshwater aquaculture in particular. In addition, *Aeromonas* spp., a ubiquitous aquatic bacterium that easily acquires antimicrobial resistance elements, can be an indicator of AMR from the aquatic environment and provide with a complementary overview of AMR in aquaculture.

This longitudinal study aims at identifying risk factors for AMR in *Aeromonas* spp. and *E. coli* from the environment of two flow-through rainbow trout farms in Brittany, France [1]. Farm A was located downstream from a wastewater treatment plant, whereas Farm B was located downstream from livestock. Both farms had a history of furunculosis outbreaks (*Aeromonas salmonicida*) and use of antibiotics. 309 samples were collected from fish, biofilm and water over the course of seven months (January to July 2020). Additionally, faecal samples of the farmers were collected. *Aeromonas* spp. and *E. coli* were isolated on GSP and EMB agar respectively; presumptive isolates were confirmed with the use of MALDI-TOF. 257 confirmed *Aeromonas* spp. and 67 *E. coli* were tested for their antimicrobial susceptibility with the broth microdilution method. The antibiotics tested were flumequine (0.016-256 µg/mL), oxalinic acid (0.016-64 µg/mL), enrofloxacin (0.016-64 µg/mL), oxytetracycline (0.016-512 µg/mL), florfenicol (0.25-32 µg/mL), trimethoprim-sulfamethoxazole (0.015/0.3-64/1216 µg/mL), and colistin (0.781-400 µg/mL). Epidemiological cut-off values were used to classify an isolate as either “wild type” or “resistant”. The percentage of AMR ranged from 13% to 60% depending on the bacterium and the antibiotic. Farm surveys were conducted in the form of questionnaires concurrently with each sampling event. Farm-level information and event-level information were collected about weather events, farm management practices, season (spring vs summer), and disease occurrence, among others. Odds ratio and 95% Confidence Intervals will be calculated to detect risk factors that can explain the presence of *E. coli* in rainbow trout farms, the presence of AMR-*E. coli*, and the presence of AMR-*Aeromonas*. Statistical analyses will be performed with the use of R Software.

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O23

Abundance and diversity of antibiotic resistance genes in different types of environmental samples: 5 years of Resistomap

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Resistomap is the first and frontrunner company for antibiotic resistance monitoring in the environment. We provide universities, research centers, governments, water utilities and industries with an end-to-end service to monitor genes associated with antibiotic resistance, mobile genetic elements, and pathogens from any type of environmental samples, including wastewater, surface water, sediment, sludge, soil, manure, stool, and others. We combine culture-independent laboratory and high-throughput gene quantification methods using the SmartChip qPCR system with our digital platform for data analysis and visualization. Since we fully operated in 2019, we have analyzed over 10,000 environmental samples across 45 countries. Figure 1 shows the medians of antibiotic resistance levels in wastewater from 28 countries. With more samples and genes analyzed, the data shown in Figure 1 could change. Besides regions, our data show that the most detected and abundant genes also differ based on the sample types. This suggests the need for a routine monitoring of antibiotic resistance in its hotspots such as wastewater treatment plants and agricultural soils, to evaluate the resistance discharge to surface waters and other environmental compartments. With routine monitoring, the data can also be used to measure the risk of resistance prevalence and spread, and as an early warning for potential outbreaks of resistant bacteria.

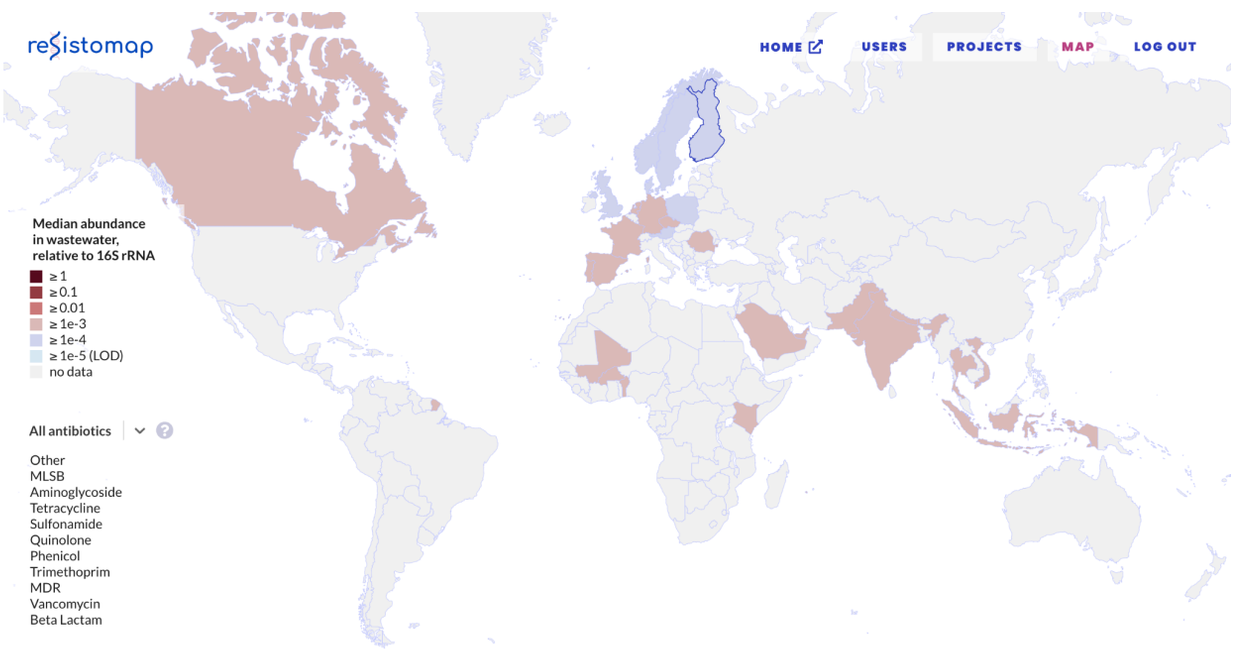

Fig. 1. Heatmap of antibiotic resistance levels in wastewater samples

O24

The evolution of the faecal resistome is shaped by age and antimicrobial use during the pig lifecycle

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Although new European Union regulations aim to restrict antimicrobial use (AMU) in livestock, antimicrobials remain an essential tool in ensuring animal health and welfare. In many countries, the pig sector represents the largest consumer of veterinary antimicrobials and thus pigs are a potential reservoir for antimicrobial resistance genes (ARG). Field studies investigating how different levels of AMU affect the evolution of antimicrobial resistance during the production cycle are lacking. This study investigated the dynamics of the porcine faecal resistome in batches of pigs on Irish farms with differing levels of AMU throughout their entire lifecycle.

Twelve Irish farrow-to-finish farms were selected according to their level of AMU in medicated feed (low, moderate, high). Ten litters of piglets were selected from each farm; pooled faecal samples were collected during each production stage (piglet, weaner, grower and finisher) and once from their dams at the initial visit. Metagenomic sequencing and read mapping against the Resfinder database was used to characterise and quantify the acquired resistome at each time point.

Genes conferring resistance to tetracyclines, macrolides, aminoglycosides and beta-lactams were the most abundant ARGs on all farms. The abundance of ARGs in growing pigs peaked during the weaner stage decreasing thereafter while the lowest abundance was observed in sows. Abundance was lower on low AMU farms compared to moderate or high AMU farms. Alpha diversity (Shannon diversity and richness) was highest in piglets and lowest in sows and finishers and was higher on the moderate and high AMU farms compared to low AMU farms. The composition of the resistome was strongly influenced by the composition of the microbiota in an age dependent manner.

To the authors' knowledge, this the first multi-farm longitudinal study to investigate the dynamics and evolution of the faecal resistome throughout the pig production cycle and gives important insight into how age and antimicrobial use impact the resistome in pig farming.

O25

Genetic analysis of ampC promotor mutants of *E. coli* from livestock exhibiting resistance to extended-spectrum cephalosporins.

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Background: Extended-spectrum cephalosporins (ESCs) are considered critically important for human clinical use.[1] There is a high focus on resistance to these antimicrobials through ESBL and plasmid-encoded AmpC (pAmpC) genes in *Escherichia coli* in both veterinary and human monitoring and surveillance programs, and many studies have focussed on these resistance mechanisms since WGS has become commonly adapted. Resistance to ESCs can also occur through mutations in the promotor region of the chromosomal *ampC* gene.[2] As this type of resistance is not transmissible on plasmids via horizontal gene transfer, the mechanism is considered a smaller risk compared to ESBL or pAmpC genes and less is known about the epidemiology of this resistance type in livestock.
Objective: To describe the epidemiology of ESC-resistant *E. coli* containing *ampC* promotor-mutants from livestock in the Netherlands using WGS.
Methods: ESC-resistant *E. coli* were selectively isolated between 2014-2021 according to EU legislation from faecal samples of broiler chickens, pigs, veal calves and dairy cattle. Isolates for which no ESBL or pAmpC genes could be detected were sequenced on Illumina NextSeq.
Results/Discussion: The proportion of ESC-resistant *E. coli* suspected to contain *ampC* promotor mutations without known ESBL pAmpC genes differs between the different livestock species. In broilers, this was 1.7% of cephalosporin-resistant *E. coli*, 5.4% in veal calves, 18.8% in dairy cattle and 35.8% in pigs.
A total of 248 *E. coli* isolates was shown to contain mutations in the *ampC* promotor region. Other commonly found mechanisms in this set of isolates include resistance to fluoroquinolones, sulfamethoxazole, trimethoprim and tetracycline, which were detected using antimicrobial susceptibility testing and were confirmed using WGS. While there was a large diversity of *E. coli* ST present in the collection, certain lineages appear to have transmitted through the production sectors.

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O26

High Throughput Screening of Antimicrobial Resistance Genes in Gram-Negative Seafood Bacteria

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Seafood and the marine environment are often considered as potential reservoirs of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs); however, there are few studies and sparse results on this sector. This study aimed at providing new data and insights regarding the content of resistance markers in various seafood samples and sources, and therefore the potential exposure to humans in a global One Health approach.

An innovative high throughput qPCR screening was developed and validated in order to simultaneously investigate the presence of 41 ARGs and 33 MGEs including plasmid replicons, integrons, and insertion sequences associated to Gram-negative bacteria.

Analysis of 268 seafood isolates from the bacterial microflora of cod (n = 24), shellfish (n = 66), flat fishes (n = 53), shrimp (n = 10), and horse mackerel (n = 115) showed the occurrence of *sul-1*, *ant(3")-Ia*, *aph(3')-Ia*, *strA*, *strB*, *dfrA1*, *qnrA*, and $bla_{\text{CTX-M-9-group}}$ genes in *Pseudomonas* spp., *Providencia* spp., *Klebsiella* spp., *Proteus* spp., and *Shewanella* spp. isolates. MGEs were identified in all bacterial species investigated. We found that the occurrence of MGE may be associated with the seafood type and the environmental, farming, and harvest conditions. Moreover, even if MGE were detected in half of the seafood isolates investigated, association with ARG was only identified for twelve isolates.

Our results corroborate the hypothesis that the incidence of antimicrobial-resistant bacteria and ARGs decreases with increasing distance from potential sources of fecal contamination. Moreover, we provide unique and original high throughput micro-array designed for the screening of ARGs and MGE in Gram-negative bacteria, which is easily implementable for monitoring antimicrobial resistance gene markers in diverse contexts.

O27

High occurrence of carbapenemase- and extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from migratory birds (*Ciconia ciconia*) with detection of high-risk clones

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Background: The presence of Enterobacterales producing carbapenemases (CP) and extended-spectrum β-lactamases (ESBL) is closely monitored in humans and animals, but the potential of migratory birds as carriers of resistance genes remains poorly understood. The aim of the study was to detect and characterize CP- and ESBL-producing *E. coli* (EC) and *K. pneumoniae* (KP) obtained from storks feeding on two landfills in Spain.

Methods: ESBL and CP-producing EC/KP were isolated from 211 stork faecal samples using chromogenic culture media, and collected isolates were sequenced using NovaSeq6000 (Illumina) and MinION (Oxford Nanopore) technologies. Resistome, virulome, sequence types (ST) and replicon profiles were determined using bioinformatic tools. Localization of ESBL/CP genes was performed using Southern blots on S1-PFGE gels.

Results: ESBL-EC/KP were detected in 71 samples (33.6%; 28.4%-EC and 5.2%-KP), while 28 samples (13.3%) contained CP-producing EC/KP (11.8%-EC and 1.4%-KP). Different sequence types (ST) (EC, n=33; KP, n=3) were identified, including high-risk clones associated with humans (EC: ST131, ST58 or ST69; KP: ST307), potential high-risk clones (EC: ST10 and ST48) and more ubiquitous clones (EC: ST46, ST155, ST117, ST617). A wide range of ESBL/pAmpC-conferring genes (*bla*$_{\text{CTX-M-1}}$/*bla*$_{\text{CTX-M-14}}$/*bla*$_{\text{CTX-M-15}}$/*bla*$_{\text{CTX-M-27}}$/*bla*$_{\text{CTX-M-32}}$/*bla*$_{\text{CTX-M-55}}$/*bla*$_{\text{CTX-M-65}}$/*bla*$_{\text{SHV-12}}$/*bla*$_{\text{CMY-2}}$/*bla*$_{\text{DHA-1}}$) was identified, as well as a large variety of CP-genes (*bla*$_{\text{KPC-2}}$, *bla*$_{\text{KPC-3}}$, *bla*$_{\text{NDM-1}}$, *bla*$_{\text{NDM-7}}$, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{VIM-1}}$ and *bla*$_{\text{GES-7}}$), including in some cases a combination of up to three types of CP (*bla*$_{\text{KPC-2}}$, *bla*$_{\text{NDM-7}}$ and *bla*$_{\text{VIM-1}}$) and/or with co-detection of ESBL/pAmpC genes. Likewise, a variety of ESBL/pAmpC- and CP-carrying plasmids were identified, such as IncY (n=30), IncF (n=60), IncX3 (n=25) IncN (n=19) or IncL (n=8). The genetic characterization of these plasmids is ongoing.

O28

Assessing the Resistome and Virulome of Human and Environmental *Escherichia coli* isolates.

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Introduction: In order to understand the dynamics of Antimicrobial Resistance (AMR), it is essential to take a One Health approach. The One Health concept recognizes the interconnectedness between human, animal and environmental health. This study aimed to assess the relationships between *Escherichia coli* isolates collected from human and environmental sources.
Methods: *E. coli* isolates (n=156) collected as part of a study investigating AMR in anthropogenically contaminated aquatic sources from 2018-2020 (n=113) were compared with those isolated from healthy participants of a cross sectional colonisation study in 2020-2021 (n=43). Total DNA was extracted from ESBL-producing *E. coli* (n = 118) and carbapenem resistant *E. coli* (n=14), and whole genome sequencing was performed on Illumina (PE150). Bioinformatic analysis was carried out to characterise isolates and to assess sequence type (MLST), phylogroup (ezclermont), resistome (Resfinder), virulome (VFDB), plasmidome (Plasmidfinder) and mobilome (Platon) of the isolates. Core Genome MLST (cgMLST) and dRep of the isolates mobilome were compared to assess similarity and clustering, with cluster hits of ≤10 allelic differences considered.
Results: In silico analysis revealed a high level of diversity, with 40 sequence types (STs) detected. The most prevalent were ST131, ST38 and ST10, which were detected in 24% (n=27), 7% (n=8), and 7% (n=8) of environmental isolates and 16% (n=7), 11% (n=5) and 11% (n=5) of human isolates, respectively. The most prevalent phylogroup was B2 (n=50), which was primarily associated with environmental isolates (n=36). The ESBL gene *bla*$_{\text{CTX-M}}$ was detected in both human (n=28, 65%) and water (n=85, 75%) isolates, while CPE remained exclusive to environmental isolates (n=14, 12%). Overall, 423 virulence factors (VF) were detected, with 83 isolates carrying 24 VF types on mobile genetic elements (MGEs). While both sample types had a median of approximately three VF, the overall proportion of isolates carrying predicted plasmid-borne VF was significantly higher in human isolates (67%) than water isolates (47%) ($p=0.028$, $\chi 2$ 4.8326). In contrast, water isolates had a higher overall number of VF ($\bar{x}$ 80.74 ± 20.33). Overall, 19 clusters were identified, of which 16 contained only environmental (n=11) or human (n=4) isolates. The remaining four clusters contained both environmental and humanisolates (n=11) all of which had the same ST, phylogroup and *bla*$_{\text{CTX-M}}$ gene detected in each.
Conclusions: The abundance of VFs and antimicrobial resistance genes, including those associated with resistance to last-resort antibiotics, in isolated *E. coli* strains suggests the potential for widespread dissemination of these genes in both aquatic ecosystems and the community.

O29

Wastewater-based Epidemiology (WBE): toward a relevant tool to monitor hospital antimicrobial resistance

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Resistance to antibiotics can lead to therapeutic failures of infectious diseases but also can slow down advances in intensive medicine or surgery. Among extremely resistant bacteria (XDR), carbapenemase-producing enterobacteria (CPE) are of particular concern because of their capacities to hydrolyze most of the β-lactam antibiotics including the last resort antibiotic carbapenems [1]. In France, *bla*OXA-48 and *bla*NDM are the most widespread carbapenemases. Another kind of XDR of concern are vancomycin-resistant Enterococci (VRE) associated with *vanA* gene [2]. Based on current knowledge, these 3 antimicrobial resistance genes (ARG) are emerging in France and spread through an epidemic mode. However, data on XDR carriage is patchy in general population because it is monitored only in high risk-hospitalized patients. New tools are needed to make surveillance of emerging resistances more comprehensive.

During SARS-CoV-2 pandemic, efficiency of wastewater-based epidemiology (WBE) has been showed [3]. Because CPE and VRE are gastrointestinal bacteria, we propose WBE to improve the surveillance in human population. To achieve this goal, we developed a strategy to monitor *bla*OXA-48, *bla*NDM and *vanA* genes in wastewater from three hospital wards. Two wards were at high-risk for XDR carriage in patients. The last one was considered at low-risk and was used as a standard. *bla*CTX-M gene was also monitored as "endemic standard" because its endemic character both inside and outside the hospital. Genes' quantifications were performed by qPCR and dPCR from hospital wastewater samples. CPE and VRE were cultured on selective media, enumerated and identified by MALDI-TOF-MS.

Molecular signals were observed both for endemic (*bla*CTX-M) and emerging ARG (*bla*OXA-48, *bla*NDM and *vanA*) with space-time variations. It was supported by significant positive correlations between results obtained by qPCR and dPCR. For the low-risk ward, a stable baseline signal was observed, with quantities of genes significantly different from those of the 2 high-risk wards. This suggests the possibility to determine a basal state of ARG quantifications in hospital wastewater to set up alert thresholds. *bla*NDM and *vanA* had epidemic dynamics as expected. Furthermore, *bla*OXA-48 signal was either similar or superior to *bla*CTX-M signal in all the wards, suggesting an undervaluation of OXA-48 diffusion in France, or a possible amplification of the signal in the hydric bacterial community of wastewater pipes. Finally, there was no taxonomic overlap between resistant bacterial communities from patients and from wastewater. These results could be in favor of ARG exchanges between patients' and waterborne bacteria.

This study highlights the feasibility of AMR monitoring with WBE in a hospital. To validate this approach, further studies had to be performed considering ARG transfers and persistence.

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O30

Metagenomic Survey of Antimicrobial Resistance in Surface Waters of Maryland with High and Low Human Impact Classifications

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Antimicrobial resistance (AMR) is recognized as one of the most critical threats to public health world-wide. In alignment with One Health strategic planning, the National Antimicrobial Resistance Monitoring System (NARMS) has added an environmental monitoring component that will focus on surface waters. Surface waters are demonstrated AMR reservoirs and key integrators across human, animal, and natural environments. Use of the National Land Cover Database of the United States Geological Survey (USGS) for sampling site selection, provides an approach by which to examine AMR by anthropogenic impact. Land use designations were used to select 30 sites representing high (n=15) and low (n=15) human impact. Dead-end ultrafiltration was used to collect 20 liters of water from each of the 30 sites and metagenomic (culture-independent) and quasimetagenomic (enriched) shotgun data were used with multiple annotation pipelines (AMRplusplus, AMRFinderplus, and CARD pipelines) to profile AMR across Maryland surface waters. Thirty-three 'critically important' ARGs (antimicrobial resistance genes) from the NARMS list were identified using quasimetagenomic data in 86% (26/30) of sampling sites. These belonged to AMR classes: Colistin (1), Macrolide (5), ß-lactam (15), and Fluoroquinolone (12). Metagenomic sequencing identified 5 critically important ARGs in 20% (6/30) of sampling sites contrasted to low impact (Figure1). This study provides a valuable baseline survey of AMR across high and low human impact classifications of Maryland surface waters and has provided a robust methodology for future monitoring efforts to support improved stewardship of antimicrobials.

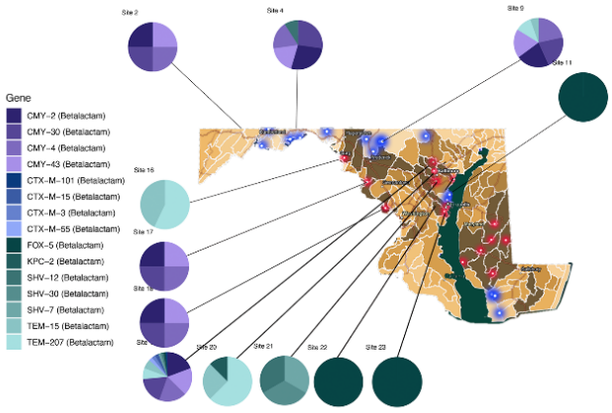

Fig. 1. ß-lactam genes in high and low impact surface waters across the state of Maryland, USA.

O31

Gram-negative bacteria resistant to last-line antibiotics from hospital and municipal wastewaters

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Wastewaters are considered as an important player in the spread of antimicrobial resistance. Here, we focused on hospital and municipal wastewaters, and river water in the city of Brno (Czech Republic) as a possible source of carbapenemase-producing Gram-negative bacteria for the environment.

Selective cultivation on antibiotic-supplemented media followed by PCR was used to obtain a set of 124 isolates carrying *bla*$_{GES}$ or *bla*$_{VIM}$ carbapenemase-encoding genes among 160 Gram-negative bacteria from hospital and municipal wastewaters, and river sampled in the Czech Republic. The isolates were species identified and tested for carbapenemase production and susceptibility to 24 antibiotics. Selected isolates were subjected to short- (Illumina) and long- (MinION) read sequencing. The obtained sequencing data were analysed for the content of resistance genes, plasmids and other genetic determinants using the BLAST algorithm and CGE tools. The phylogenetic tree including publicly available genomes of *Enterobacter asburiae* was conducted using Prokka, Roary and RAxML.

The beta-lactamase GES was produced by 121 strains of different genera (*Enterobacter, Escherichia, Citrobacter, Klebsiella*, etc.). The predominance of the *bla*$_{GES-1}$ (51%, no carbapenemase activity) and *bla*$_{GES-5}$ (49%, carbapenemase activity) variants was observed among 68 sequenced isolates. The isolates carried resistance genes to other antimicrobial groups such as aminoglycosides, fosfomycin, sulphonamides, etc. The conjugation transfer of *bla*$_{GES}$ was successful only in 18% of isolates and was mainly associated with ColE2- like plasmids (n=28).

Three VIM-1-producing *E. asburiae* strains originated from hospital and municipal wastewaters. The *bla*$_{VIM-1}$ gene was located within class 1 integron on different types of plasmids including IncFIB(K)/IncFIB(pQil), IncFIB/IncFII and one non-typeable plasmid. One of the isolates carried *mcr-10* conferring resistance to colistin while in another isolate *mcr-9* gene without the colistin resistance phenotype was detected. In addition, phylogenetic analysis of 360 publicly available *E. asburiae* genomes was performed, showing high genetic diversity but also high prevalence of carbapenemases, alerting the increasing importance of this species for human health.

The dissemination of Gram-negative bacteria with clinically important resistance via wastewaters was observed. These findings pointed out important contribution of hospitals and community wastewaters in transmission of multi-drug resistant pathogens with resistance to last-line drugs.

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O32

One-Health AMR spread in a French Caribbean island

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Antimicrobial resistance (AMR) is considered as a major health issue. AMR needs to integrate different ecosystems such as humans, animals and the environment, called the “One Health” approach. It remains knowledge gaps in this field, particularly concerning the environmental side, among which is the role of environmental conditions (exposome, diversity of the matrices and their microbiota) on the transmission of AMR. Furthermore, the impact of touristic activities in a specific geographical area has not been fully addressed. In this context, the ACRAS-R project aims to characterize the transmission pathways of AMR in a continuum from the clinical settings to the natural receiving environment in the French Caribbean island, Guadeloupe, and assess the contribution of the exposome and tourism on AMR spread. Four sampling campaigns have been done in Guadeloupe during wet and dry seasons. Clinical isolates from patients were collected in addition to water samples (hospital wastewater, influent and effluent of wastewater treatment plants (WWTPs) and their receiving environment), animal feces and the contents of aircraft toilets from metropolitan France to Guadeloupe. DNA extraction for microbiome and resistome analysis was performed on these samples as well as genomic/plasmid analysis on a selection of 3GCsusceptible and ESBL-producing Escherichia coli. The exposome was also analyzed. Preliminary results showed a prevalence of 7% of ESBL E. coli in clinical samples, whereas the prevalence in environmental samples was 32%. We showed the variations in terms of resistome in the different sampling sites, revealing a wide diversity of resistance genes to antibiotics, heavy metals and mobile genetic elements, with a higher relative abundance of these genes in hospital water samples. Concerning aircraft toilets, the relative abundance of resistance genes was variable depending on the company and the day of sampling, but we observed the same
resistome/microbiome signature (proportional abundance). The most abundant phyla in water samples were the same as those usually found in the human gut microbiota: Firmicutes, Bacteroidetes and Actinobacteria, illustrating the anthropogenic impact. In animal samples, the main phyla were Firmicutes, Proteobacteria and Bacteroidetes. Exposome analysis revealed the presence of antibiotic residues (Sulfamethoxazole, Ciprofloxacin, …) mostly in hospital samples and biocides (Levamisole, Pyrantel pamoate, ...) in WWTP. The results collected will help develop a mathematical model to predict resistance and understand the transmission routes of AMR.

Session 3 - Mechanisms and dissemination of antimicrobial resistance in animal and zoonotic pathogens

O33

Klebsiella a hitherto underappreciated zoonotic pathogen expressing AMR phenotypes of importance to *One Health*

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The threat posed by multidrug resistant (MDR) members of the genus *Klebsiella* can be magnified due to the convergence of antibiotic resistance and virulence phenotypes. These bacteria are now responsible for severe community (CA) - and hospital (HA) - acquired human infections and increasing reports have epidemiologically linked members of this genus to animal infections and within the broader environment. Furthermore, bacterial isolates have been cultured from niches in all three axes of One Health; humans, animals and the environment.

This presentation will review the current understanding of the taxonomy of the genus *Klebsiella*, using comparative genomics, in addition to commenting on a recent study characterising a collection of MDR *K. pneumonia* cultured from a nosocomial setting. The nature of the associated AMR-encoding genotypes will be described along with the virulence factors in these isolates. Furthermore, data commenting on the prevalence of *Klebsiella* species isolated from food and food-producing animals, a hitherto infrequently recognised ecological niche, will be explored. Finally in the context of the environment and *One Health*, the impact that commonly used agrochemicals, such as the herbicide glyphosate, exerts on the antimicrobial susceptibility profiles of these bacteria and associated mechanisms of resistance will be presented. This paper will highlight the relevance of contextualising genomic data to extend our understanding of the risks posed to *One Health*, associated with emerging *K. pneumoniae* and how predictive genomic data can be used to inform and interpret surveillance information.

O34

WGS-based characterization of emerging pathogenic OXA-48-producing *Enterobacter hormaechei* belonging to ST114 and ST418 in a Swiss companion animal clinic

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Background. *E. hormaechei* producing the carbapenemase OXA-48 was repeatedly identified in infections of companion animals hospitalized at a Swiss veterinary clinic, where an outbreak with OXA-48-producing *K. pneumoniae* occurred in the previous months.
Objectives. To determine the genetic relatedness of the *E. hormaechei*, and assess the possible acquisition of mobile genetic elements from *K. pneumoniae*.
Methods. Hybrid assemblies for phylogenetic and comparative analysis of the isolates (n=9) collected between May 2021 and August 2022 from infectious material of 5 dogs and 3 cats treated at a veterinary hospital in Switzerland were obtained by sequencing with Illumina, PacBio and Oxford Nanopore Technologies. Antimicrobial susceptibility testing was performed by broth microdilution. Phylogenetic relatedness and molecular characteristics were analyzed using publically available bioinformatic tools (e.g., Unicycler v.0.4.8 for hybrid assemblies) and web platforms, such as the Center of Genomic Epidemiology for plasmid and resistance-gene typing, and JSpeciesWS for average nucleotide identity (ANI) calculation, as well as software included in seqSphere v7.7.5 (Ridom) and Geneious v2023.03 (Dotmatics) for the development of a custom core-genome MLST (cgMLST) scheme and core-genome SNP analysis, and for read-mapping and (whole-genome and whole-plasmid) alignments, respectively.
Results. The strains were identified as *E. hormaechei* subsp. *xiangfangensis* belonging to sequence type (ST) 114 (n=6) and ST418 (n=2), and as *E. hormaechei* subsp. *hoffmannii* of ST78 (n=1). Core-genome SNP analysis confirmed the clonality of the ST114 and ST418 isolates (0 to 10 SNPs). The strains contained genes associated with decreased susceptibility to meropenem ($bla_{\text{OXA-48}}$), cephalosporins ($bla_{\text{TEM-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{SHV-12}}$, $bla_{\text{DHA-1}}$, bla_{LAP}), aminoglycosides (*aac(6')-Ic-cr5*, *ant(2'')-Ia*, *aadA*, *aph(3'')-Ib*, *aph(3')-Ia*, *aph(6)-VIb*), tetracyclines [*tet*(D)], phenicols (*catA2*, *catB3*, *floR*), quinolones (*oqxAB*, *qnrA*, *qnrB*, *qnrS*), trimethoprim (*dfrA*), sulfonamides *(sul1*, *sul2*), macrolides [*mph*(A)], fosfomycin (*fosA*), and rifamycin (*arr*). Five ST114 strains carried a 250 kb IncHI2 multidrug resistance plasmid harboring the colistin resistance gene *mcr9.1*, but were phenotypically susceptible to colistin, as already observed for this species. All strains harbored the $bla_{\text{OXA-48}}$ on identical 63 kb IncL plasmids, but one ST114 and both ST418 isolates showed an inverted variant of the $bla_{\text{OXA-48}}$-carrying transposon Tn*1999.2*. This specific transposon was also present in the plasmids carried by the *K. pneumoniae* clones previously responsible for an outbreak in the same animal clinic. Additionally, the $bla_{\text{DHA-1}}$-carrying IS*CR1* element located on 140 kb IncFIB plasmids in one ST114 and in both ST418 clones was identical to that found in the *K. pneumoniae* outbreak strains, suggesting the transfer of the complete element to an endogenous plasmid backbone.
Conclusion. OXA-48-producing *Enterobacter hormaechei* has emerged in companion animals in Switzerland. Common ecological niches could favor the spread of plasmid-borne carbapenemases among *Enterobacterales* and the emergence of MDR *E. hormaechei* clones.

O35

Massive plasticity of IncHI2-ST3 plasmids encoding clinically important IMP-4 in bacteria from silver gulls

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Even though the transmission of carbapenem-resistant *Enterobacteriaceae* (CPE) is mostly associated with clinical facilities, the number of reports of the CPE dissemination outside of healthcare settings is increasing [1]. Mobilization of *bla*IMP-4 and other resistance genes via plasmids was observed in a silver gull colony during our previous study [2]. The most prevalent group, IncHI2 plasmids, showed changes in size during conjugation transfer. In this study, we focus on microevolutionary changes of large IncHI2 plasmids of sequence type 3 (IncHI2-ST3) carrying *bla*IMP-4 that are prone to rearrangements and fusions with other plasmids during a horizontal transfer.

One *Klebsiella pneumoniae* and 42 *Escherichia coli* isolates carrying IncHI2-ST3 and -ST3-like plasmids encoding IMP-4 carbapenemase were short-read sequenced. Out of these, 21 representative wild-type isolates and 12 transconjugants carrying plasmids of diverse size compared to the original plasmid, were selected for long-read sequencing. Complete plasmids were reconstructed and deep bioinformatical analyses on the plasmids were performed.

All studied IncHI2-ST3 and -ST3-like plasmids provide resistance to multiple antibiotics (e.g. carbapenems, beta-lactams) and non-antibiotic resistance determinants (e.g. heavy metals). IncHI2-ST3-like plasmids carry additional genes, e.g. encoding heat resistance or transporters, compared to IncHI2-ST3 plasmids. Analysed plasmids tend to change size through the horizontal transfer during which they acquire genes for ethanol utilization, ATP synthesis, conjugative transfer, virulence, and resistance to antibiotics, heavy metals and formaldehyde. Conjugation of HI2 plasmids lead to fusion with other plasmid types (IncX5, IncP1, IncF-type). The fusions were driven by homologous recombination of MGEs.

Studied IncHI2-ST3 are highly variable, dynamically changing plasmids that are linking resistance to last-line carbapenems with other antimicrobials. Their plasticity represents a threat to public health due to the ability to cointegrate with other plasmids and incorporate additional genes. The changes we observed may provide novel traits beneficial for their bacterial hosts, leading to persistence and further spread of AMR.

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O36

Determining the impact of veterinary antibiotics sub dosage on resistance transfer in animal gut microbiota - a One-Health approach

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Background
Antimicrobials are an important global resource and have been widely used over the years. From treating humans and animals to ensuring livestock production. However, excessive usage of antibiotics has been driven by pressure and development of bacterial resistance worldwide. Resistance-associated genes can be genetically transmitted and exchanged between pathogenic species co-habiting environments, human and animal communities. Our aim was to evaluate whether sub dosage of widely administered antibiotics in veterinary medicine could enhance plasmid transfer, and consequently, resistance genes exchange in animal gut microbiota.
Materials
Escherichia coli carrying pOXA-48a (bla_{OXA-48}) plasmid was submitted to minimum inhibitory concentration for ceftiofur, enrofloxacin, florfenicol, tiamulin, colistin, neomycin, amoxicillin, erythromycin, apramycin, oxytetracycline, narasin, lincomycin, sulfamethazine, zinc oxide, and copper. Treatments in sub-inhibitory dosage were performed with and without antioxidant (edaravone), before moving to i) the conjugation assays to access conjugation frequency; ii) RT-qPCR to access the genomic expression of SOS response associated genes; iii) and fluorescence ROS detection assay to access stress oxidative response. Statistical analyses were performed using Graph Prism 9.3.1.
Results
Increased conjugation frequencies were observed when treating the isolate with florfenicol (13.7x higher, $p = 0.0172$) and oxytetracycline (97.2x higher, $p < 0.0001$) compared to the control. Increased expression of the SOS-associated *recA* gene for both treatments was also observed (florfenicol, 2,20 ± 0,61, $p = 0.0057$; oxytetracycline, 1.24 ± 0.16, $p < 0.05$). The ROS experiment reinforced previous results for both antibiotics with an intensity of fluorescence 3.7x (florfenicol, $p < 0.001$) and 2.5x (oxytetracycline, $p = 0.0009$) higher when compared with the control. Addition of edaravone reduced plasmid conjugation frequencies when supplemented with the inducing antibiotic (florfenicol, 7.6x lower, $p = 0.0251$; oxytetracycline, 2.4x lower, $p = 0.0002$).
Conclusions
Induction of resistance dissemination through sub-inhibitory dosage seems to be a key factor in terms of public-health control. In the current One Health concept, we believe that environment, animals, and human health are interlinked and exchanging genetic determinants, mainly critical antimicrobial resistance. Our findings reinforce the commitment to this concept, detecting at least two widely used antibiotics in veterinary medicine, oxytetracycline, and florfenicol, as horizontal resistance genes transfer enhancers.

O37

A *In silico* Analyses of Incompatibility Group (Inc) HI2 Plasmids from Enteric Bacteria

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Antimicrobial resistance (AMR) plasmids play key roles in the dissemination of resistance genes among bacterial pathogens. One interesting group of resistance plasmids is the incompatibility (Inc) HI2 plasmids which are typically large in size (>200 kb), often encode for AMR, heavy metal resistance (HMR) and disinfectants/biocide resistance (DBR) and can be transmissible potentially facilitating resistance dissemination among enteric bacteria. To better understand the distribution and diversity of AMR, DBR and HMR-encoding genes among the IncHI2 plasmids, we utilized multiple computational approaches to evaluate resistance elements and transfer-associated genes among plasmid sequences. A total of 667 IncHI2 plasmid sequences (all available at the time of the search) were identified using the IncHI2 replicon sequence[1] for a BLAST searching against "Complete Plasmids" available in the Microbial Genomes database. FASTA files for each plasmid were extracted from GenBank and analysed using AMRFinderPlus[2] web-based tools to detect various resistance genes, IntegronFinder[3] to identify class 1 integrons and Plasmid Transfer Factor database to identify transfer (type 4 secretion system, T4SS) genes within the plasmid sequences. Among the plasmid population the most common genera identified as carrying the plasmids include *Enterobacter* (N=209), *Escherichia* (N=208), and *Salmonella* (N=204) isolated across the globe. In many cases, the distribution of the AMR and HMR genes were diverse with plasmids from *Escherichia* and *Salmonella* showing similarity in comparison to the *Enterobacter* and other taxa, which group ed together. For the HMR, the plasmids from *Enterobacter* and other taxa, had a significantly higher ($p<0.05$) prevalence of many of the mercury resistance genes (*merA,B,D,E,G,P,R,T)* and the arsenic resistance gene, *arsC,* compared to those from *Escherichia* and *Salmonella.* For the AMR genes, there was also diversity, for example, among the sulfonamide resistance genes, *sul1* was more common among the plasmids from *Enterobacter* and other taxa, while *sul2* and *sul3* were more common among those from *Escherichia* and *Salmonella.* Similar diverse AMR genetic trends were displayed for several of the other antimicrobial classes as well. Integron sequences were common among the plasmids and most plasmids contained most IncHI2-asssociated T4SS genes. These findings provide insights into the diversity of resistance genes among bacteria and allow for the understanding of the dissemination of AMR across different enteric bacterial taxa and geographical locations. The results also underscore the value of computational-based approaches for the assessment of AMR and mobile genetic elements, which are important to understand the molecular epidemiology and public health impact of these large plasmids.

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O38

Investigation of *rmtB*-harbouring *Salmonella* and *Escherichia coli* from a pig farm

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Aminoglycosides are antibiotics with broad-spectrum and rapid bactericidal activity making them critically important in human and veterinary medicine as recognized by World Health Organisation (WHO) and World Organization for Animal Health (WOAH), respectively. However, the usage of these antibiotics in humans, livestock, and companion animals has led to the emergence of resistance with post-transcriptional methylation of the 16S rRNA prompting greatest concerns. Methylation of bases key in the binding between 16S rRNA and aminoglycosides leads to decrease or loss of affinity of the antibiotic to its target, thus resulting in high-level resistance to many clinically important aminoglycosides[1]. A frequently detected 16S rRNA methylase gene in Enterobacteriaceae isolates is *rmtB,* however this gene is not common in bacteria of veterinary origin. This study aimed to investigate and characterise the spread of *rmtB*-harbouring Enterobacteriaceae isolates from environmental samples on a pig farm as well as to determine the effect of disinfection to mitigate risk of bacterial spread within the farm. Two visits were carried out, where samples were collected between farm cleaning and disinfection procedures. Short- and long-read whole-genome sequencing (WGS) was used to analyse recovered isolates to investigate mobility and spread of *rmtB*-harbouring element(s) in *Salmonella enterica* and commensal *Escherichia coli*. Amikacin resistant isolates were found at both farm visits suggests that disinfection efforts were not effective in removing resistant bacteria. WGS analysis identified a stable ~9kb sequence containing *rmtB* to be chromosomally located in resistant *S. enterica* isolates and on IncFII type plasmids in resistant *E. coli* isolates. Furthermore, this sequence contained insertion (IS)26 element, which is known to promote dissemination of *rmtB*[2], thus posing a risk of *rmtB* to be spread horizontally to other strains and species. *rmtB* emergence in a livestock environment highlights the need for aminoglycoside resistance to be closely monitored.

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O39

Various mobile genetic elements involved in the dissemination of phenicol-oxazolidinone resistance gene *optrA* in *Streptococcus suis*

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Oxazolidinones represent the last-resort antimicrobial agents against infections caused by multidrug-resistant Gram-positive pathogens. Antimicrobial resistance to phenicol and oxazolidinone (PhO) by the dissemination of *optrA* gene leads to treatment failure in both veterinary and human medicine. However, genetic mechanisms for the dissemination of *optrA* gene in streptococci was limited, especially for the zoonotic pathogen *Streptococcus suis*. In this study, we observed a rapid increase of PhO resistance in *S. suis* is attributable to the transferable resistance gene *optrA* (from ~2.5% to 56.4% between ~2011 and 2021) in 601 isolates from eastern China. we selected *optrA*-positive *S. suis* isolates for whole genome sequencing and analysis. IS*1216E* element was presented in 85% of the *optrA*-carrying contigs despite genetic variation observed in the flanking region. IS*1216E-optrA*-carrying segments could be inserted into larger mobile genetic elements (MGEs), including integrative and conjugative elements (ICEs), plasmids, prophages and antibiotic-resistance-associated genomic islands. IS*1216E*-mediated circularization occurred to form the IS*1216E-optrA*-carrying translocatable units, suggesting a crucial role of IS*1216E* in *optrA* spreading. Three types of *optrA*-carrying MGEs (ICE, plasmid and prophage) were successfully transferred via conjugation at different transfer frequencies. In addition, conjugative transfer of *optrA*-carrying plasmid and prophage in streptococci was validated for the first time. In conclusion, different *optrA*-carrying MGEs are associated with the rapid increase of PhO resistance in *S. suis*. Considering the abundance of MGEs in *S. suis* and the mobility of IS*1216E-optrA*-carrying translocatable units, attention should be paid to the potential risks to public health by the emergence and spread of PhO-resistant *S. suis*.

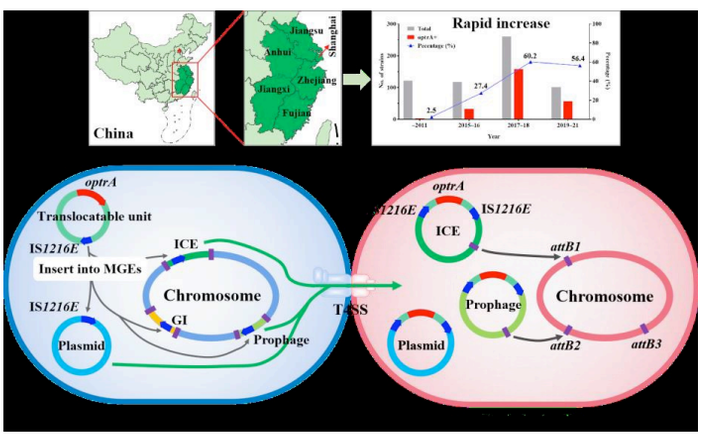

Fig. 1. Transfer of *optrA*-carrying MGEs contributes to the rapid increase of PhO resistance.

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O40

Development of efflux pump MexB and MexY dual inhibitors against muliti-drug resistant *Pseudomonas aeruginosa*

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Multi-component type (RND type) multidrug efflux pumps are important in the multidrug resistance of Gram-negative bacteria. However, despite efforts to develop efflux inhibitors, clinically useful inhibitors are not available at present. This study is aimed to develop drugs for multi-drug resistant *Pseudomonas aeruginosa* (MDR-PA) infections targeting the drug efflux pump. We focused on two RND-type multidrug efflux pumps, MexB and MexY, and decided to discover a dual inhibitor against both pumps (Fig. 1). We have discovered an advanced compound that demonstrates the potent and dual inhibition activities both MexB and MexY and enhances the antibacterial activity of the combination antimicrobial agent against *P. aeruginosa* through rational structure-based drug design. The combination of drug efflux pump inhibitors can restore the antimicrobial activity of various chemotypes of antimicrobials and is expected to be highly effective in the treatment of MDR-PA infections. In addition, the drug efflux pump inhibitor inhibits the efflux of the antimicrobials and increases its concentration in the bacteria, and thus is likely to contribute not only to the recovery of antimicrobial activity but also to the prevention of resistance to the antimicrobials itself. Furthermore, since RND-type multidrug efflux pumps is involved in the resistance mechanism of not only *P. aeruginosa* but also many Gram-negative bacteria, the findings from this development of the drug efflux pump inhibitors can be applied to the development of the efflux pump inhibitors against several drug-resistant bacteria.

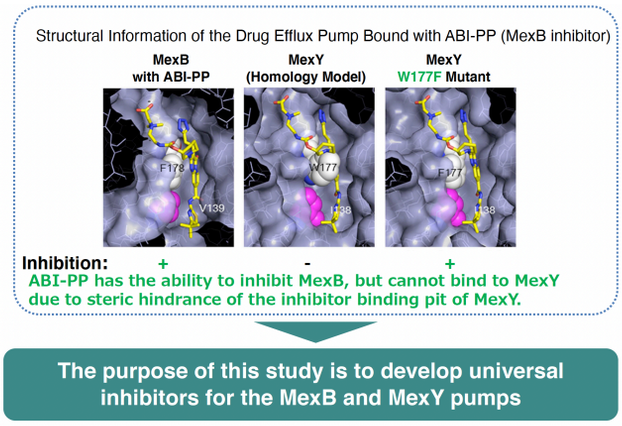

Fig. 1. Development of Universal Inhibitors for the MexB and MexY Multidrug Efflux Pumps in *P. aeruginosa*.

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O41

Glyphosate Induces Changes to Cellular Metabolic Pathways and Selects for Antimicrobial Resistance in *Klebsiella* species.

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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 (1).

In this study, the minimum inhibitory concentration (MIC) of glyphosate was determined by broth microdilution for five *Klebsiella* isolates (including the type strain MGH 78578), along with clinical (*K. pneumoniae* and *K. variicola)*, environmental (*K. variicola)*, and an animal (*K. variicola*) strain. 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. Changes in protein expression in the presence of sub-inhibitory glyphosate were assessed by LC-MS/MS.

When the *Klebsiella* species were exposed to glyphosate, a significant ($P < 0.05$) increase in resistance to tigecycline was recorded in all study isolates, and to imipenem in all but one strain (a *K. pneumoniae* strain with a RamA deletion). PM results describing bacterial metabolism (carbon, nitrogen, phosphorous and sulphur utilisation), chemical sensitivity and effects of pH, osmosis, and ionic effects were studied. showed that metabolic pathways were affected following exposure to glyphosate leading to reduced metabolism of carbon sources and redirecting flux towards the purine salvage 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. Preliminary proteomic results appear to confirm the emphasis on purine metabolism.

This study reports 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.

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O42

Quinolone resistance and expression of RND efflux pump in *Stenotrophomonas* spp. under exposure to sub-lethal concentration of nickel

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The worldwide use of antibiotics in human and animal medicine and in food animal production has contributed to the emergence of antibiotic resistant bacteria. Anthropogenic activities have participated to the continuous input of antibiotics, antibiotic resistant bacteria and antibiotic resistance genes into aquatic and terrestrial environments. Along to antibiotics, toxic chemical stressors like metals might be present in contaminated environments and exert a co-selection pressure that contributes to the maintenance and proliferation of antibiotic resistance in the environment (1). A positive relationship between antibiotic and metal resistance has been described in *S. maltophilia* soil strains (2). This species is responsible for healthcare-associated infections and has the ability to resist numerous antibiotic families due to efflux pumps. However, regardless of the origin of the strains (clinical or environmental), the efflux pump content is similar (3) to that of *S. indicatrix*, a phylogenetically related species never isolated from humans (4).

To evaluate whether the exposure to anthropogenic pressures could lead to the evolution and the induction of the emergence of antibiotic resistance in an environmental species, representing a risk to human health, we performed a phylogenetic study in the *Stenotrophomonas* genus using two environmental strains, a *S. maltophilia* one and a *S. indicatrix* one. We then conducted an experimental evolution assay comparing both strains under sub-MIC $NiCl_2$ exposure every two days for one month. Throughout the experiment, ~1100 isolates were collected and submitted to antibiotic phenotyping. The results showed an increase in the number of quinolone resistant colonies of *S. indicatrix* (MIC > 2 mg/L and MIC > 4 mg/L for ciprofloxacin and pefloxacin respectively). We screened for the gene expression of 8 Sme efflux pumps and showed an overexpression of *sme*VWX gene from day 9 in *S. indicatrix* strain exposed to MIC/8 of $NiCl_2$. All these data suggest the potential for emergence of efflux-mediated antibiotic resistance among environmental bacteria exposed to metals, even if they are only slightly available in the environment, and consequently the risk for humans to be in contact with them.

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O43

Heavy metals may increase ARG persistence in the environment

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The OneHealth approach requires determining the risk that antibiotic resistance genes (ARGs) in the environment pose to human health. ARGs in the environment provide opportunities for human microbiome bacteria and human pathogens to acquire resistance to antibiotics through horizontal gene transfer. Heavy metals can impose a selective pressure on environmental bacteria and select for antibiotic resistance, since ARGs can co-occur with metal resistance genes. In addition, crossresistance mechanisms between metals and antibiotics such as efflux pumps have been observed. Since metals persist longer than antibiotics in the environment, they may exert a selective pressure on environmental bacteria for longer periods than antibiotics and could cause an increase in the persistence of ARGs in the environment. In our study, river water microcosms were exposed to heavy metals (copper and zinc), fluoroquinolone antibiotics (ciprofloxacin and ofloxacin) and both for 30 days to evaluate the persistence of ARGs using shotgun metagenomic analysis of the extracted DNA. Higher abundances of efflux pumps conferring resistance to ciprofloxacin and ofloxacin were observed in samples exposed to metals, both in the presence and absence of antibiotics than in controls. Therefore, copper and zinc could select for and maintain fluoroquinolone resistance in the environment by cross-resistance. The ARGs that showed an increase in abundance in heavy metal-contaminated river water depended on whether antibiotics were present or not. Therefore, both metals and antibiotics conferred resistance, although the resistance profiles were not the same. Thus, our results are consistent with heavy metal persistence leading to the maintenance of ARGs in the environment.

Session 4 - Understanding the connection of antimicrobial resistance between Animals and Humans

O44

WGS-based relationship between multidrug-resistant bacteria from humans and animals: emphasis on CPE and MRSA

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The increasing number of bacteria exhibiting resistance the clinically important antibiotics in humans, animals and environment has raised questions about their origin and their dissemination between the different settings. Whole genome sequencing (WGS) is nowadays the technology of choice, which permits to rapidly characterize and compare bacterial strains from different settings. In this presentation, the degree of relatedness and genetic characteristics of carbapenemase-producing Enterobacterales (CPE) from companion animals and methicillin-resistant *S. aureus* (MRSA) from animals was determined and the resulting WGS-based data were compared with those of strains from human origin.

During the summer 2018, a major outbreak of *Escherichia coli* ST410 producing the carbapenemase OXA-181 occurred in a veterinary clinic in Switzerland, where one quarter of the hospitalized companion animals acquired this bacteria during their stay at this clinic [1]. Over the same period, one employee of the clinics was found to be colonized with the same *E. coli* [2]. Nevertheless, CPE were not detected among owners of CPE positive animals [3]. In the following years, OXA-48-producing and multidrug-resistant *Klebsiella pneumoniae*, *Enterobacter cloacae* and *E. coli* also emerged in infection sites of companion animals in the same clinic. The emergence of CPE in veterinary settings raised the question whether they are related to the strains causing infections in humans in Switzerland. Human OXA-181 and OXA-48 producing CPE were made available for comparative genomic analysis from the National Reference Center for Emerging Antibiotic Resistance (NARA), University Fribourg, where human clinical CPE isolates from Switzerland are sent for biochemical and molecular analysis and archived [4].

Cases of human infections with the livestock-associated MRSA (LA-MRSA) ST398 were recorded in Switzerland in the past years. Comparative genomic analysis with LA-MRSA ST398 from different animals including pigs, horses, cattle, poultry, companion animals as well as veterinarians (See Poster of Fernandez *et al.*) revealed that the human clinical strains were related to strains causing infections in horses clustering into a specific lineages. They were also detected in veterinary personnel and in non-hospitalized healthy horses [5]. The LA-MRSA ST398 of this lineage also share the same resistance and virulence profile.

Acquisition of CPE and MRSA in veterinary clinics poses the evident risk of their further dissemination to other animals, humans and into the environment. However, the direction of transmission between the different settings remains an open question and is likely to occur in both direction. A continuous One-Health and WGS-based surveillance will contribute to rapidly identify new emerging multidrug-resistant bacteria and their potential reservoirs and routes of dissemination in animals, humans and environment.

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O45

U.S. FDA's National Center for Toxicological Research AMR Research: Applications across the One Health Continuum

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Research scientists at the U.S. FDA's National Center for Toxicological Research (NCTR) have engaged in multiple antimicrobial resistance-related research projects and several of these projects address "One Health" needs within the FDA. This presentation provides an overview of key efforts to generate data which can inform these public health efforts. The projects focused on 1) the characterization of antimicrobial resistance (AMR) genotype and phenotypes in food, feed and companion animals; 2) the development and use of in vitro systems to evaluate factors that potentially contribute to antimicrobial and pathogenicity in enteric bacteria; and 3) the development of database and associated bioinformatics tools to analyze whole genome sequencing (WGS) data to characterize pathogens, including *Salmonella enterica* and *Escherichia coli* that occur throughout the One Health sphere. The exposure of bacterial populations to antimicrobials can contribute to the emergence of AMR in various ecosystems due to selection of resistant mutants and/or the acquisition of AMR genes through horizontal transfer. Research efforts at NCTR have examined plasmids and their role in the dissemination of AMR and virulence genes among different bacteria. The linkage of AMR and virulence is important, since often the more severe manifestations of enteric diseases require antimicrobial therapy and may allow bacteria to adapt in diverse environments across the One Health continuum. Specific efforts have examined *Salmonella*-associated plasmids, including the use of WGS and PCR-based methods to characterize multidrug resistant (MDR) strains of *Salmonella* from a variety of food animal and human clinical sources to understand the distribution of AMR genes in the different bacterial populations [1-3]. Research has also centered on the emergence of extended-spectrum β-lactamases (ESBLs) and carbapenem-resistant *E. coli* isolated from companion animals as a potential public health issue[4,5]. Many of these resistance and virulence gene determinants in *Salmonella* and *E. coli* are co-located on plasmids that contribute to drug resistance and pathogenicity. Because of the need for better characterization of factors that contribute to the dissemination of AMR, research efforts at NCTR have also addressed extrinsic factors that contribute to resistance transfer, such as the impact of an antimicrobial exposure on plasmid transfer. Our studies showed a diversity of responses to the exposures; however, in several cases, exposure to different concentrations of chloramphenicol and tetracycline increased plasmid transfer among bacterial isolates. To further understand such selective transfers, a plasmid transfer gene database and analyses tools were developed to evaluate the transfer-associated genes that can facilitate the dissemination of plasmids among bacteria (see presentation by Dr. Jing Han at this conference). Overall, research efforts at NCTR have led to a better understanding of mechanisms associated with AMR in various host environments and factors that contribute to the dissemination of AMR across the One Health continuum.

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O46

Investigating the circulation of AMR between animals, humans and their environment in Cambodia - ARCAHE

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Antimicrobial resistance (AMR) is an increasing public health concern and threatens decades of infectious disease control efforts. The emergence and spread of AMR are mainly attributed to the overuse of antibiotics, whether in humans, animals, or the environment. The development of AMR control and monitoring strategies has become a priority in Low- and Medium-Income Countries (LMICs), particularly in South East Asia, where very high levels of AMR prevalence were reported. Cambodia is also confronted with AMR. We observe the emergence of bacteria resistant to multiple antibiotics, which directly impact human health. Today, we still do not know why and where AMR emerges and how it circulates between humans, animals, and the environment in Cambodia. This information is nevertheless essential for the establishment of effective control strategies. In the framework of the FSPI ARCAHE project (Antibiotic resistance at the Human/Animal/Environment interface in a "One Health" approach in Cambodia), we sought to study, in a "One Health" approach, the resistome present in the environment and animals of patients infected with multi-drug resistant *Enterobacteriaceae*. The research was conducted utilizing genomic and metagenomic sequencing strategies and through the extensive use of bioinformatics workflows.

Patient samples were collected at the provincial hospital of Battambang. Environmental samples (e.g., soil, wastewater, drinking water) and oral/rectal swab samples from domestic animals (chickens, pigs, ducks, and dogs) were collected at the patient households. In total, 995 samples were collected. Each sample was collected in duplicate; one for bacterial cultures with antibiotic-selective media and one for metagenomics analysis.

The *Enterobacteriaceae* strains isolated from patients, animal, and environmental samples, have been submitted to Whole Genome Sequencing (WGS) on the Illumina NovaSeq platform (MGX platform, Montpellier, France). For analyzing the WGS datasets, we have implemented a bioinformatics workflow: Bacterial Assembly and Antimicrobial Resistance Genes detection In Nextflow (*baargin*). This tool allows us to assemble the genomes, check for contamination, detect the sequences of plasmid origin, identify the ARGs and mutations conferring resistance, annotate the genomes, and perform a pangenome analysis of all the datasets provided to the workflow. *Baargin* outputs presence/absence matrices that enable comparative study between the different strains based on the resistance profiles.

The comparison between the bacterial resistance profiles of strains isolated from animal and environmental samples collected at the patients' households shows that the animal resistance profiles, such as Extended-Spectrum Beta-Lactamases producing *Enterobacteriaceae* (ESBLE) or Carbapenemase Producing *Enterobacteriaceae* (CPE), were more similar to the patient resistance profiles than the environmental ones. These results suggest similar antibiotic or biological pressure and/or privileged circulation between humans and animals.

O47

The effect of antibiotic usage on resistance in humans and food-producing animals: a longitudinal, one-health study using European data

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This paper estimates the effect of antibiotic usage in humans and food-producing animals on the incidence of resistance in zoonotic bacteria in both humans and animals. We use comprehensive data on usage in humans and food-producing animals of 11 antibiotic classes and occurrence of resistance in three bacterial species common in humans and food-producing animals from European surveillance reports over 11 years in 31 countries. This allows us to make numerous contributions to the growing literature on antibiotic use and resistance.

First, although antibiotic usage occurs simultaneously in humans and other animals, existing studies have almost exclusively considered the effect of antibiotic use in humans *or* other animals. Our study introduces an analysis with usage in both humans and food-producing animals, allowing us to identify the marginal effect of usage in humans and animals. We are also able to estimate their joint effect.

Second, our longitudinal data allow us to estimate the effect of usage on resistance in a causal framework, rather than just estimating correlations. We use a methodology pioneered in the economics literature that allows us to bound the causal effect from use to resistance, though not to estimate it precisely.

Third, we show the effect of antibiotic usage in humans on resistance in food-producing animals at an ecological level. To our knowledge, no previous study has attempted to show how antibiotic use in humans is related to resistance in other animals.

The estimated effects are both substantial and statistically significant. Strikingly, the lower and upper bounds of the effect of antibiotic use in animals on resistance in humans are not smaller than the effect of antibiotic use in humans. The estimated elasticities are, from the perspective of long-term impact on resistance, disturbingly large. Even at the lower bound, an increase in antibiotic use in animals of only 10% is expected to increase the prevalence of resistance in animals by around 2% and in humans by around 0.3%. Since, as we show in a recent paper, resistance tends to persist over a period of years, increases in usage may lead to long-term increases in resistance.[1]

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O48

Comparative genomics of *Escherichia coli* ST131 of human, animal and environmental origin from the Czech Republic

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Escherichia coli and its multidrug-resistant sequence types (ST) have been found to be particularly successful at spreading antibiotic resistance. One of the most widespread clones of medical importance is ST131 which is responsible for human nosocomial diseases such as urinary tract or bloodstream infections. This clone is also capable of infecting animals and disseminating through various environments and its sublineages have been also linked with retail meats. The aim of the study was to explore the genetic structure of a large collection of source diverse ST131 in terms of antibiotic resistance genes and plasmids and perform the phylogenetic analysis to evaluate the clonality of isolates coming from different sources.

To this end, 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 years 2009-2021. Isolates were assigned to STs and the content of antibiotic resistance genes, virulence factors and plasmid replicons was assessed. Phylogenetic analysis based on the core-genome of all ST131 isolates, in combination with characterization of *fimH* and *bla*CTX-M genes, enabled categorization of strains into clades A, B, C0, C1 and C2.

Overall, we detected 72.4% of isolates carrying a variant of *bla*CTX-M and 19.8% of isolates carrying a variant of *bla*TEM responsible for resistance to beta-lactam antibiotics regardless of the clade or source. Most isolates belonged to clade C (804/898), characterized by *fimH*30 variant, with C1 (29.2%; 262/898) carrying *bla*CTX-M-27 and C2 (58.8%; 528/898) linked with *bla*CTX-M-15. This clade frequently contained isolates with point mutations in *parC* and *gyrA* responsible for fluoroquinolone resistance. Forty-nine isolates belonged to clade A carrying *fimH*41 and *bla*CTX-M-27, while 45 isolates were assigned to clade B with *fimH*22 and *bla*CTX-M-14. Inside clade C1, high clonality was observed among a subset of isolates originating from humans, wild birds, and wastewater (0-50 SNPs difference); a trend also observed in clade C2 within groups of isolates obtained from humans and wastewater. Presence of diverse F-type plasmids was detected throughout all isolates regardless of clade association. Plasmids similar to pUTI89 (F29:A-:B10) that carry *cjrABC-senB* cluster contributing to virulence in pathogenic bacteria from urinary tract infections, were associated with clade A isolates from humans and water samples. Clade B isolates frequently carried ColV plasmids of various sequence types and originated mainly from humans, wild birds, and poultry.

Our collection of *E. coli* ST131 comprising isolates from various sources, environments and years was mostly clonal, particularly in phylogenetic groups C1 and C2. Most of the isolates were associated with diverse epidemiologically relevant F plasmids disseminated throughout various niches. These findings highlight the importance of studies focused on previously unmapped parts of the world and support the claim of the global threat these bacteria pose.

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O49

Understanding the risks associated with recirculating aquaculture systems: persistence of antimicrobial-resistant *Enterococcus* spp. and comparison with isolates from human infections

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Antimicrobial resistance (AMR) is recently becoming a concern in the aquaculture sector. In rainbow trout farms from Portugal, Novais et al. (2018) found that inflow water and commercial feed were vehicles of AMR-*Enterococcus*. Enterococci are considered indicators of fecal contamination from birds and mammals, and indicators of AMR from the environment.
With this longitudinal observational study, we aim at monitoring the presence and persistence of *Enterococcus* spp. in a recirculating aquaculture system (RAS) raising rainbow trout at Oniris (Nantes, France). We also aim at determining AMR phenotypes and at comparing with isolates from human infections. Over five monthly sampling events (February-July 2022), 95 samples were collected from all inputs and compartments of the RAS: water (n=33), biofilm (n=23), sediment (n=5), fish feces (n=23) and feed administered to fish (n=4). Water was sampled at each treatment step: arrival of city water, sand filtration, biological filtration, UV treatment, arrival at fish tanks and reservoir tank. Suspected colonies isolated on Slanetz and Barley agar (37°C, 48h) were confirmed with the use of MALDI-TOF. Ten samples out of 95 were positive for *Enterococcus* spp. In fish feed, *Enterococcus faecium* was found (1 sample out of 4), as well as *E. gallinarum* (2/4) and *E. casseliflavus* (1/4). Oppositely, uncommon species such as *E. moraviensis, E. silesiacus* or *E. termitis* were found in RAS samples: 3 samples out of 33 (9%) in water, 3/23 (13%) in fish feces, 1/23 (4%) in biofilm and 2/5 (40%) in sediment. The highest prevalence was observed in May (50%) followed by July (20%), whereas *Enterococcus* spp. were not detected in February. It is remarkable that the RAS water temperature increased over time (14˚C to 18˚C). These results suggest that *Enterococcus* introduced by feed do not persist in a RAS raising rainbow trout. All *Enterococcus* isolates, in addition to 20 *E. faecium* from human infections retrieved from the University Hospital (CHU), Nantes, will be tested with the broth microdilution method against Vancomycin, Teicoplanin, Quinupristin-Dalfopristin, Tetracycline, Daptomycin, Ciprofloxacin, Erythromycin, Tigecycline, Linezolid, Gentamicin, Ampicillin and Chloramphenicol. These results will shed light on the similarity between AMR phenotypes in *Enterococcus* spp. from the RAS environment and human infections.

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O50

Successful host adaptation of IncK2 plasmids.

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Antimicrobial resistance is a global health threat and because it is often encoded on plasmids, it is crucial to understand the dynamics of plasmid spread and host adaptation. It was shown that IncK plasmids can be divided into two separate lineages named IncK1 and IncK2. IncK2 plasmids are found predominantly in poultry. The relatively high body temperature of chicken influences IncK2 plasmids fitness cost, copy number and stress response in the *Escherichia coli* host. These data shed a light on IncK2 plasmid's success and persistence in *E. coli* of chicken origin.
This study analyzed 50 IncK2 carrying isolates of human, poultry, cattle, pig and environmental origin from 10 European countries and Lebanon, as well as 14 publicly available IncK2 plasmid sequences. IncK2 carrying isolates analyzed in this study were sequenced using both Illumina and Nanopore technology. A phylogenetic analysis of all plasmids was performed in order to determine the genetic relatedness of IncK2 plasmids isolated in different countries and from different sources. Additionally, a genome wide association study (GWAS) was performed on annotated sequences to assess if an association exists between specific genetic features and various sources, that could explain the suspected IncK2 plasmid adaptation to the chicken host. The obtained results show that an antitoxin for the Hok/Gef family protein and YdeA protein were predominantly found on plasmids isolated from chicken and therefore are significantly associated with IncK2 from the chicken host. Moreover, protein YffA is significantly associated with IncK2 from human.
In combination with prior findings, this study shows that adaptation of plasmids to a chicken host is a complex process that involves both physiological and genetic determinants. Understanding the basis of plasmid adaptation may lead to the development of intervention strategies that reduce the spread of AMR plasmids between different sources.

O51

Indications of transmission of *mcr*-1.26 IncX4 plasmids along the poultry food chain to humans

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Antimicrobial resistance is one of the major global health challenges. The drivers of emergence and evolution of antimicrobial resistances include antimicrobial use and abuse in human, animal and environmental sectors. Their interconnection can contribute to the spread of resistant bacteria and resistance determinants between sectors, inevitably affecting the health of these contiguous habitats. The association of resistance determinants with mobile genetic elements facilitates transmission across habitat boundaries further aggravating the problem. One such example is the transmissible plasmid-mediated colistin resistance (*mcr*) first discovered in 2016. Since then, the main determinant *mcr*-1 has been found in a vast variety of plasmid backbones in diverse bacterial species across all sectors. To date, 34 variants of *mcr*-1 have been described with varying prevalence rates. Whereas common variants, such as *mcr*-1.1 can be used for quantification of transmission events, rare variants allow for epidemiological tracing-back analysis to identify the origin and transmission dynamics of these genes. *mcr*-1.26 is rare and was detected in 2018 in an *E. coli* isolated from a hospitalized patient in Germany. We report on the presence of *mcr*-1.26 in 16 *E. coli* and one *K. pneumoniae* originating from poultry, such as feces and retail meat, already found in 2014. The *mcr*-1.26 was located on transmissible IncX4 plasmids. Comparative bioinformatics analysis suggest horizontal transfer of a conserved *mcr*-1.26 IncX4 plasmid type within poultry husbandry and demonstrate a high similarity to the plasmid reported for the human sample. We further identified two novel *mcr*-1.26-IncX4 types. Three *E. coli* carried an *mcr*-1.26-IncX4 plasmid that acquired an additional resistance gene (bla_{TEM}). Notably, in addition to the bla_{TEM} gene, the *mcr*-1.26-IncX4 plasmid of the *K. pneumoniae* isolate encoded a further transposase.
Based on the temporal occurrence and high similarity of the plasmids between poultry and human isolates, our study provides first indications for poultry husbandry as the primary source of *mcr*-1.26 and its transmission along the poultry food chain to humans. We highlight the transmission of *mcr*-1.26-IncX4 between Enterobacterales to acquire resistance to colistin, a last-line antimicrobial in human medicine. Finally, our study indicates ongoing plasmid evolution of *mcr*-1.26-IncX4 in different bacterial hosts by the acquisition of an additional beta-lactam resistance gene and a transposase.

Session 5 - Novel approaches, methods and tools dedicated to antimicrobial resistance (detection, evolution, diagnostics, surveillance)

052

The present and future in antibiotic resistance surveillance

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Antibiotic resistance is a major, global health issue which is now recognized as a major cause of deaths worldwide. Especially, resistance to beta-lactam in Gram-negative bacilli such as Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is driving a significant part of this burden. Surveillance of antibiotic resistance is crucial to undertake preventive and mitigation measures. Surveillance be applied to different levels of the microorganisms (gene, mobile genetic element, clone), in different sectors (human, animal and environment), in different settings (community, healthcare structures, farms, wildlife, polluted or pristine environments), times and places. Hence, surveillance shall be driven by the needs and the actions taken beyond. In the last decade, major advancements have been achieved in the field of sequencing. Especially, we have now the possibility to finely reconstruct genomes and mobile genetic elements and to track them with a high precision. Sequencing technologies together with bioinformatic tools and knowledge databases also enable the analysis of the antibiotic resistance gene contents (the resistome) from a large diversity of samples and thereby provide knowledge about the circulation of antibiotic resistance gene between pathogens and their environment. Here, we will present how the application of sequencing technologies can be applied to the surveillance of multidrug resistant bacteria in healthcare structures and in a One Health perspective. Then, we will also see how metagenomic sequencing improved our knowledge about the gut resistome and how it can be used to clinical samples.

O53

An in-house 45-plex array for the detection of antimicrobial resistance genes in Gram-positive bacteria

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Identifying antimicrobial resistance (AMR) genes and determining their occurrence in Gram-positive bacteria provide useful data to understand how resistance can be acquired and maintained in these bacteria. We describe an in-house bead array targeting AMR genes of Gram-positive bacteria and allowing their rapid detection all at once at a reduced cost. A total of 41 AMR probes were designed to target genes frequently associated with resistance to tetracycline, macrolides, lincosamides, streptogramins, pleuromutilins, phenicols, glycopeptides, aminoglycosides, diaminopyrimidines, oxazolidinones and particularly shared among *Enterococcus* and *Staphylococcus* spp. A collection of 124 enterococci and 62 staphylococci isolated from healthy livestock animals through the official Belgian AMR monitoring (2018–2020) was studied with this array from which a subsample was further investigated by whole-genome sequencing. The array detected AMR genes associated with phenotypic resistance for 93.0% and 89.2% of the individual resistant phenotypes in enterococci and staphylococci, respectively. Although linezolid is not used in veterinary medicine, linezolid-resistant isolates were detected. These were characterized by the presence of *optrA* and *poxtA*, providing cross-resistance to other antibiotics. Rarer, vancomycin resistance was conferred by the *vanA* or by the *vanL* cluster. Numerous resistance genes circulating among *Enterococcus* and *Staphylococcus* spp. were detected by this array allowing rapid screening of a large strain collection at an affordable cost. Our data stress the importance of interpreting AMR with caution and the complementarity of both phenotyping and genotyping methods. This array is now available to assess other One-Health AMR reservoirs.

Classes	Antimicrobials	Targeted genes
Aminoglycosides	Kanamycin[a] [1] (KAN) Gentamicin[b] (GEN) Streptomycin[c] [1] (STR)	*aadD*[a], *aadE*[c], *aacA-aphD*[ab], *aphA3*[a], *aph2-Id*[ab], *aph2-Ie*[bc]
Glycopeptides	Vancomycin (VAN)	*vanA*, *vanB*, *vanC*$_1$, *vanC*$_{2\text{-}3}$
Lincosamides	Clindamycin [1] (CLN)	*ermA*, *ermB*, *ermC*, *lsaA*, *lsaE*, *lnuA*, *lnuB*
Macrolides	Erythromycin (ERY)	*ermA*, *ermB*, *ermC*, *mefA/E*, *mphC*
Oxazolidinones	Linezolid (LZD)	*cfr*, *optrA*, *poxtA*
Phenicols	Chloramphenicol (CHL)	*catp*$_{C194}$, *catp*$_{C221\text{-}223}$, *cfr*, *fexA*, *optrA*, *poxtA*
Pleuromutilins	Tiamulin [1] (TIA)	*cfr*, *lsaA*, *lsaE*, *vgaA*, *vgaB*, *vgaD*,
Streptogramins	Quinupristin (group B-streptogramin) / dalfopristin (group-A-streptogramin) (Synercid, SYN)	*Streptogramin A (dalfopristin): lsaA, lsaE, vatA, vatB, vatC, vatD, vatE, vgaA, vgaB, vgaD, cfr* *Streptogramin B (quinupristin): ermA, ermB, ermC, lsaA, lsaE, vgbB*
Diaminopyrimidines	Trimethoprim [1] (TMP)	*dfrA/C*, *dfrD*, *dfrK*, *dfrG*
Tetracyclines	Tetracycline (TET)	*poxtA*, *tetO*, *tetK*, *tetL*, *tetM*

Fig. 1. List of the AMR genes targeted by the array and their related antimicrobials and antimicrobial classes monitored by broth microdilution in *Enterococcus* spp. and/or *Staphylococcus* spp. [1] Antimicrobials monitored by broth microdilution in *Staphylococcus* spp. only. [1]

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O54

Identification of novel antibiotic resistance integron gene cassettes in water samples with different anthropogenic levels

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Mobile genetic elements (MGEs) are essential vehicles for stockpiling and disseminating antibiotic resistance genes (ARGs), allowing their transfer from non-pathogenic (environmental or commensal) to pathogenic bacteria. This strong association of ARGs with MGEs pushes us to explore the diversity of genes associated with mobile elements. This could allow us to recover novel ARGs with no sequence similarity with previously known ARGs. Classes 1-3 mobile integrons are known to host many ARGs as gene cassettes (GCs), being major spreaders and a promising source to search for novel ARGs [1]. Moreover, they display many GCs of unknown function (gcu) that could account for novel ARG activity, as has been previously reported [2].

To further explore the diversity of mobile integron GCs, we developed a novel *cassettomics* strategy combining metagenomics and bioinformatics approaches that allows us to (i) determine the relative proportion of each integron GC within a given sample and (ii) locate to which integron class these GCs are associated, and (iii) identify novel integron GCs. To do this, we took advantage of the 5'/3' conserved regions to specifically amplify each integron class coupled with a specific pipeline, which also allows us to enrich the samples in integrons to access novel GCs which are scarcely represented. This approach has previously allowed us to identify in 20 samples 593 novel GCs, many from class 3 integrons, including some experimentally verified novel ARGs.

Using our *cassettomics* pipeline with a list of 1078 known GCs, we detected more than 150 novel GCs (~4% of them closely related to known ARGs) from 4 water samples with different anthropogenic levels (urban, animal, and littoral settings), and with a threshold of 90% of nucleotide identity. Moreover, 25% of them encode proteins with already known domains with different biochemical activities and 60% proteins of unknown function, which might also be involved in antibiotic resistance. We have developed a filtering and selection workflow based on genetic and epidemiological features to choose the best candidates from these GCs of unknown function in order to perform functional analyses.

Such preemptive antibiotic resistance (AR) detection, using different high throughput sequence-based strategies, allows the detection in the environment of novel ARGs which could be the future superbugs.

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O55

Determination and quantification of microbial communities and antimicrobial resistance on food through host DNA-depleted metagenomics

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Humans are exposed to microorganisms from animals via food, including antimicrobial resistant (AMR) microorganisms. It is difficult to quantify the number of antimicrobial resistant microorganisms on food using traditional culture methods due to the large number of possible microorganisms that can be found on food. Metagenomics may be used to identify AMR genes on food, but directly sequencing food metagenomes is inefficient as DNA from the host (animal or plant) vastly outnumbers the microorganism DNA present. We optimised a host DNA depletion enabling efficient sequencing of food microbiota, thereby increasing the proportion of non-host DNA sequenced 13-fold (mean; range: 1.3-40-fold) compared to undepleted samples. The method performed best on chicken, pork and leafy green samples which had high mean prokaryotic read proportions post-depletion (0.64, 0.74 and 0.74, respectively), with lower mean prokaryotic read proportions in salmon (0.50) and prawn samples (0.19). We show that bacterial compositions and concentrations of (AMR) genes differed by food type, and that salmon metagenomes were influenced by the production/harvesting method. The approach described in this study is an efficient and effective method of identifying and quantifying the predominant bacteria and AMR genes on food.

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O56

Characterization and quantification of antibiotic resistance gene variants in the gut microbiota

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The world is reaching a point where the effectiveness of antibiotics could be completely compromised in the near future, if antimicrobial resistance (AMR) continues to spread globally. The intestinal microbiota of human and domestic animals is suspected to be the main reservoir of AMR bacteria and therefore of antibiotic resistance genes (ARGs) [1]. Advances in DNA sequencing technologies and more specifically metagenomic approaches have shown that several ARGs are prevalent and shared between most gut microbiota [2, 3]. Although nucleotide diversity is documented for well-known ARGs, the specific distribution of each ARG variant in gut resistomes is still completely unknown. This study aims to understand if ARGs described as identical in different microbiomes are actually the same variants.

We adapted DESMAN [4] algorithm previously developed to reconstruct bacterial strain genomes from metagenomic data, to characterize ARG variants from a pool of ARGs. This tool was applied to ARGs detected in metagenomic cecal samples of chickens raised under two different conditions. Among 22 dominant ARGs analyzed, 15 have at least 2 stable variants and 7 (*ant*(6), $bla_{\text{TEM-1}}$, *erm*(B), *erm*(F), *tet*(Q), *tet*(L) and *tet*(32)) have variants showing different proportions between cecal metagenomes depending on rearing condition and age of animals. Sequence comparison between ARG variants reconstructed by the DESMAN tool and those identified from bacterial isolates revealed a perfect match. These results attest to the reliability of this tool to reconstruct different variants of the same ARG directly from metagenomic data and to infer their relative proportions in different samples. It opens the way for further analyze the relative abundance of ARG variants in metagenomic datasets and finally deciphering their transmissions between microbiomes of different hosts.

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O57

Development of an *in vitro* biofilm model for the study of the impact of fluoroquinolones on sewer biofilm microbiota

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Introduction. Sewer biofilms are likely to constitute hot spots for selecting and accumulating antibiotic-resistant bacteria and genes. The objective of this study was to optimize culture conditions to obtain a stable and reproducible *in vitro* biofilm, mimicking the biofilm collected in sewers, in order to study the impact of fluoroquinolones on sewer biofilm microbiota.

Materials and methods. Biofilms were produced on coupons in CDC Biofilm Reactors®, continuously fed with nutrient (R2A broth) and inoculum (1/100 diluted wastewater), at 21°C. Different culture conditions were tested: (i) initial inoculum: diluted wastewater with or without sewer biofilm, (ii) coupon material: concrete *vs* polycarbonate, and (iii) time of culture: 7 days (d_7) *vs* 14 days (d_{14}). Biofilms were collected on d_7 and d_{14}. Once the growth conditions have been determined, the impact of fluoroquinolones (ciprofloxacin and norfloxacin) on sewer biofilm microbiota, alone or in combination, was evaluated.

Results. 16S rRNA gene and bacteria quantification revealed that the biomass was the highest when *in vitro* biofilm was formed on concrete coupons. 16S rRNA gene sequencing and analysis revealed that the addition of sewer biofilm to the initial inoculum and that the material coupon did not affect the taxonomic diversity. However, the diversity increased on d_{14} for all tested conditions. The taxonomic composition of environmental samples differed: *Campylobactereles* dominated in wastewater, whereas they were in minority in the sewer biofilm. *In vitro* biofilms were dominated by *Enterobacterales*. Quantification of *qnr*A, B, D and S genes showed that the relative abundance of these genes was higher in *in vitro* biofilms than in sewer biofilm and wastewater. The following growing conditions were chosen: concrete coupons, initial inoculation with sewer biofilm and a time culture of 14 days. Administration of fluoroquinolones had no impact on the abundance of *qnr* genes, except for *qnr*A genes when both fluoroquinolones were administered concomitantly. Sequencing of *gyr*A and *par*C genes was performed to determine the proportion of *E. coli* resistance mutations. Exposure to fluoroquinolones led to the increase in the proportion of mutations in *gyr*A (codons S83L and D87N) and in *par*C (codon S80I).

Conclusion. This study allowed determining the culture conditions to develop an *in vitro* model of sewer biofilm. This model should allow for identifying concentrations of antibiotics and antibiotic cocktails in water that promote the selection and dissemination of resistance. Eventually, this work could help define threshold concentrations of antibiotics that exert selection pressure on environmental microbiota for monitoring purposes.

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O58

From genotype to antimicrobial resistance (AMR) phenotype prediction: how genomic proficiency tests can facilitate the AMR laboratories' performance evaluation

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Background: The transition from traditional microbiology to whole genome sequencing (WGS) based diagnostics has been emerging for the last decade in clinical settings, with the expansion for benchtop WGS platforms. Hence, the need to translate, understand and assess the predicted antimicrobial resistance (AMR) phenotype, based on bioinformatics analysis of WGS data, is gaining ground progressively. Prediction of AMR phenotypes is multifactorial and depends among others on the employed WGS platform, bioinformatic tool as well as scientific expertise for data evaluation. Thus, the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR) recently launched a genomic proficiency test (GPT) with the objective to assess the ability of the network's participating laboratories to detect AMR determinants based on *in silico* data applying own bioinformatic tools as well as the ability to infer an AMR phenotypic profile based on the predicted determinants.
Methods: Clinically relevant bacterial species, such as *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp., *Enterococcus* spp. and *Staphylococcus aureus* were included in the 2021 and 2022 GPT iterations; GPT-2021 and GPT-2022. Two strains of three bacterial species were dispatched to the participating laboratories as both live cultures and DNA. Participants were requested to perform WGS followed by bioinformatic analysis to detect AMR determinants (genes and chromosomal mutations) and predict the inferred AMR phenotype, for a selected panel of antimicrobial compounds. Results were submitted to an online reporting and evaluation tool, where proficiency was scored against references results, generated from closed genomes of the same cultures assessed, using ResFinder [1] and PointFinder [2].
Results: Twenty-eight and twenty laboratories from Europe participated in GPT-2021 and GPT-2022 iterations respectively. A high concordance (>90% correct results) to the reference data were observed in the laboratories' ability to detect AMR genes from the provided DNA (63% and 70% in GPT-2021 and GPT-2022, respectively) and live cultures (68% and 75% in GPT-2021 and GPT-2022, respectively), despite the differences observed in the applied WGS methodologies and bioinformatics tools. Thus, indicating that the applied WGS methodologies and bioinformatics tools generated reliable results. For chromosomal mutations, >90% correct results were submitted by 86% and 55% of the laboratories (both live cultures and DNA), for GPT-2021 and GPT-2022 respectively. Regarding the laboratories' ability to infer phenotypic AMR profiles, 85% of the participants (live culture) and 90% (DNA) for GPT-2022 as well as 36% of the participants (live culture) and 29% (DNA) in GPT-2021 obtained >90% correct results.
Conclusion: The majority of laboratories that participated in GPT-2021 and GPT-2022 (16/18 and 14/14 respectively, for live culture/DNA analysis), obtained a satisfactory overall performance, with >90% correct results. Participation in the DTU GPT, assists the EURL network laboratories to pinpoint flaws in the WGS workflows and /or in the *in silico* AMR identification tools, contributing to provide reliable data for surveillance as well as for control and prevention measurements. A successful *in silico* AMR phenotype prediction based on WGS data is, however, still heavily dependent on the scientific evaluation of the data outputted by the AMR detection tools.

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O59

Correlation analysis of bacterial cell morphology and multidrug resistance

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It has been widely known that cell morphology in Gram-negative rods may change when exposed to stresses such as antimicrobial agents, and that once bacteria acquire antimicrobial resistance (AMR), they no longer undergo significant morphological changes after exposure[1,2]. On the other hand, the morphology of drug resistant bacteria in the absence of exposure is still lacks knowledge, even though it is a subject of great interest in understanding the phenomenon of AMR.
We acquired phase-contrast optical microscopy images of multidrug-resistant *Escherichia coli* obtained by evolutionary experiments[3] under non-exposure condition, and quantified the cell outline information as 10 types of features. The similarity between drug susceptible and resistant bacteria was examined using the histogram crossover method. Then, using Weighted Gene Correlation Network Analysis (WGCNA), we identified a group of genes highly correlated with the morphological characteristics of the resistant bacteria.
We revealed that resistant bacteria have morphological characteristics that are quantitatively different from susceptible bacteria, and that these differences are particularly large in resistant bacteria generated using quinolones and β-lactams. We also identified 56 genes that correlated well with morphological characteristics of resistant bacteria, and GO (Gene Ontology) enrichment analysis revealed that xenobiotic transmembrane transporter activity is significantly enriched for this gene cluster. Furthermore, these analyses revealed that *soxRS* may be one of the key regulators in clarifying the relationship between AMR and morphology.
Our finding provides new insights into the morphological changes of Gram-negative rods during the AMR acquisition process, and indicates the potential for future development of AMR spread prevention methods that utilize morphological information.

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Session 1 - Roles of the environment in resistance evolution and transmission

P1

Detection of resistance genes and resistant bacteria in wild birds from the French Antarctic and Austral Territories

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Antimicrobial resistance (AMR) in wild birds most likely reflects the contamination of their natural environment, in which the selective pressure of antibiotics is supposed to be very weak, with antimicrobial resistance genes (ARGs) from anthropogenic sources. AMR acquisition in wild birds most probably occurs through foraging (scavenging, predation), but the exact source of AMR in specific wild individuals is often difficult to infer. The aim of this study was to determine the AMR load in fecal samples of wild birds living on islands of the French Antarctic and Austral Territories, which is a highly pristine environment with limited human presence.

Cloacal and fecal samples of wild birds (n=346, mostly form king penguins *Aptenodytes patagonicus* and lesser sheathbill *Chionis minor*) were collected in 2021 from Crozet, Kerguelen and Amsterdam islands using e-swabs™. From each sample, bacteria were isolated on selective ChromID ESBL and non-selective ChromID CPS agar (Biomerieux) and total DNA was extracted (Bacterial DNA kit, Macherey-Nagel). Isolated bacteria were identified by Maldi-TOF and antimicrobial susceptibility was tested by disc-diffusion. High-throughput microfluidic real-time PCR amplification of 23 ARGs associated to gram-negative bacteria was performed using 48.48 dynamic arrays (Biomark™; Fluidigm, USA) on total DNA extracts after a pre-amplification step.

No extended-spectrum beta-lactamase (ESBL) producing isolate was identified on selective plates, while 546 different bacteria were collected on non-selective plates, including 182 *Enterococcus* spp and 73 *Escherichia* spp isolates. Antibiograms revealed very few resistance phenotypes. All tested ARGs, except *mcr-1* and *tetA*, were detected at least in one sample. The most frequent ARGs were those conferring resistance to aminoglyocosides (aph(3')-Ia, n=311; *strA* n=169; *strB*, n=190), sulfonamides (*sul1*/*sul2*, n=144/167) and tetracyclines (*tetB*, n=115). The class 1 and class 2 integrons were respectively found in 147 and 286 samples. ESBL genes $bla_{\text{CTX-M-1-group}}$ and $bla_{\text{CTX-M-9-group}}$ were sporadically identified (n=25 and n=54). Within Possession Island, in Crozet Archipelago, the proportion of resistance genes decreased with distance from the scientific station.

Our results give further evidence that ARGs have now percolated in the most pristine environments. Direct plating of the samples, without any enrichment step, did not allow the isolation of ESBL-producing isolates, even though ESBL genes could be identified using HT-qPCR. This indicates that the AMR burden is still low and restricted to the sub-dominant flora. Interestingly, our results showed that the proportion of ARGs decreased with distance from the scientific station, suggesting the role of anthropization in the spread of ARGs. A new sampling campaign is currently being conducted to provide more evidence for this hypothesis.

P2

Majority of multi-drug resistant *Aeromonas* isolates from the urban-impacted Akaki river carried enteric toxins

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The spread of antibiotic-resistant and virulent bacteria through aquatic environments is a global public health challenge (1). *Aeromonas* species are emerging gastrointestinal pathogens that are rapidly becoming antibiotic resistant (2). This study characterized *Aeromonas* isolates from an urban-impacted river that runs through Addis Ababa in Ethiopia. *Aeromonas* spp. were isolated from five sites, chosen based on anthropogenic activities along the Akaki river during dry and wet seasons. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion and carbapenemase production was confirmed by a hydrolysis test. Polymerase chain reaction and whole genome sequencing were employed for molecular characterization. From a total of 163 *Aeromonas* isolates, 144 were implicated in human infections (*Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas veronii*) and their resistance patterns were assessed. Majority of the isolates were resistant to amoxicillin (144, 100%), ampicillin (142, 98.6%), amoxicillin/clavulanate (117, 81%), imipenem (65, 45.1%), ertapenem (71, 49.3%), and cefotaxime (86, 59.7%). The rate of resistance to the tested antibiotics varied between species, where most of *A. veronii* (37/49, 75.5%) and *A. hydrophila* (28/33, 84.8%) were resistant to imipenem but none of *A. caviae*. The resistance prevalence of *A. veronii*, *A. hydrophila,* and *A. caviae* to meropenem was 63.3%, 57.6%, and 4.8%, respectively. Resistance to carbapenem in most *A. hydrophila* (85.6%) and nearly all *A. veronii* was mediated by carbapenemase production. A significant association of resistance was observed with season of isolation for some antibiotics tested. In these isolates, 6 toxin genes were found. Cytotonic enterotoxin (*alt* and *ast*) and hemolysin (*hlyA*) were detected in the majority of *Aeromonas* isolates. Cytotoxic enterotoxin (*act*) gene was found only in *A. hydrophila* and *A. veronii*, whereas none of *Aeromonas* isolates carried Shiga toxins (*stx-1* and *stx-2*). Of the 21 *Aeromonas* isolates that were sequenced, nearly all in the current study were new sequence types. Isolates from farthest upstream and downstream river sites contained fewer antibiotic resistance genes that belonged to the β-lactamases. Presence of bla_{cphA} gene was associated with the phenotypic carbapenem resistance. These results highlight that river in megacities maybe a reservoir of virulent, multi-drug resistant and carbapenemase-producing *Aeromonas*.

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P3

Assessment of antimicrobial resistant Enterobacterales from the dairy production environment in low and high zinc containing areas

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Antimicrobial resistance (AMR) is a critical public health concern and it is acknowledged that a One Health approach is required to address it effectively. Limited information is available on the dissemination AMR in the primary food production environment, where the presence of heavy metals may play a role in promoting AMR gene transmission. In Ireland, where agriculture is of key importance, soils are very well mapped in relation to the levels of heavy metals they contain. This includes zinc, which naturally occurs in low and high concentrations across the country.

Therefore, the objective of this study was to evaluating the presence of AMR Enterobacterales in dairy pasture soil and bovine milk filters on farms in high and low zinc areas across Ireland.

Samples were collected from two distinct areas across Ireland, with varying zinc concentrations. Enterobacterales were enumerated from soil and bovine milk filter samples and the presence of ESBL- producing Enterobacterales (ESBL-PE), carbapenem resistant Enterobacterales (CRE), and ciprofloxacin resistant Enterobacterales (FQR-E) were assessed on selective agars. Suspected colonies were identified by Maldi-TOF and antimicrobial susceptibility testing (AST) was performed on confirmed Enterobacterales. Additionally, chemical analysis was conducted on soil samples.

A variety of AMR Enterobacterales of differing antimicrobial susceptibility were isolated from both sample types. This study demonstrated that the primary food production environment can harbour AMR Enterobacterales, which might enter the food chain and cause a risk for human health.

P4

Resistance profiles of viable airborne antibiotic-resistant bacteria from antibiotic-free broiler operations

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Introduction. Animal production represents a reservoir for antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARG). The absence of selective pressure in antibiotic-free productions is thought to reduce the prevalence of ARB in the farm environment. Confined farming activities are also known to produce high concentrations of bioaerosols. These air-suspended biological particles can contain ARB. Bioaerosols can be emitted outside through building ventilation systems and disseminate ARB into the surrounding environment where their impact remains unknown. Our main objective was to characterize the resistome of bacteria present in the indoor air of antibiotic-free broiler operations using selective culture approaches. **Methods.** Two antibiotic-free broilers operations in the province of Quebec, Canada, were sampled once. Airborne culturable bacteria were collected with a high flow rate liquid type air sampler (SASS® 2300: Research International). The liquid air samples were inoculated both on solid media and in broth enrichment with antibiotics in order to recover ARB. According to their colony morphology, randomly selected isolates were identified with MALDI-TOF/MS (Bruker Daltonics) and 16S rRNA sequencing. Whole-genome sequencing (WGS) (NovaSeq6000, Illumina) was performed on twelve isolates and ARGs were predicted using the Comprehensive Antibiotic Resistance Database. **Results.** 36 bacterial species were retrieved from the two farms. Isolates sent to WGS were: *Acinetobacter baumannii*, *Aerococcus viridans*, *Enterobacter cloacae*, *Enterococcus faecium, Escherichia coli*, *Globicatella sulfidifaciens*, *Klebsiella pneumoniae*, *Pseudomonas monteilii*, *Rothia nasimurium*, *Staphylococcus saprophyticus*, *Stenotrophomonas* spp. and *Streptococcus alactolyticus*. This latter had resistance genes associated with plasmids, *msrA* (macrolide), *spd* (aminoglycoside), *dfrD* (diaminopyrimidine) and *lnuA* (lincomasimde). *R. nasimurium* harbored *IsaE*, a gene previously found in porcine MRSA isolate and *vatE* a transposon-mediated gene found in *E. faecium.* Genes conferring resistance to aminoglycosids (*AcrD*, *AAC(3)-Via, ANT(3'')-IIa*), beta-lactam (*ompA*), carbapenems (*OXA*-317) and cephalosporins (*ADC-159, TEM-135*) were found in *A. baumannii, E. coli* and *K. pneumoniae* had *E. cloacae* had a gene conferring resistance to bacitracin. Additionally, antibiotic susceptibility testing will allow comparison of the resistance genotypes and phenotypes. **Conclusion.** Even in the absence of selective pressure during animal production, viable ARB harboring genes conferring resistance to multiple antibiotics are present in the indoor air of two antibiotic-free broiler operations. The same resistome monitoring methodology will be applied to broiler operations administrating antibiotics. This comparison will serve to understand how livestock management approaches can aim to mitigate problems associated with antimicrobial resistance.

P5

Seasonal Dispersion of Antimicrobial Resistance Genes in Aerosols: A One Year Monitoring at the puy de Dôme summit

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The issue of antimicrobial resistance and its spread in the environment has become a major concern in recent decades. Carried by bacteria, antimicrobial resistance genes (ARGs) are found in many surface ecosystems (soils, rivers and oceans) and the atmosphere could be a major transport route. The atmospheric system is wide and dynamic and the bacteria it contains (~10^3 cell.m^{-3} of air) fluctuates in both space and time; season is a critical factor of this variations.

In this work, airborne ARGs and their seasonal fluctuations were assessed between July 2016 and August 2017 at the puy de Dôme meteorological observatory (1465 m a.s.l., France). Sampling consisted in PM10 aerosols collection over one-week periods at high flow rates (60 $m^3.h^{-1}$) on pre-calcinated quartz filters (Ø 150 mm). In total, 48 aerosol samples were retrieved and analyzed for the presence of 34 different subtypes of ARGs from the main existing classes of antibiotic resistance: quinolones, β-lactams, macrolides, tetracyclines, sulfonamides, aminoglycosides, and vancomycins, including one mobile genetic element. These were further related to airborne bacterial concentration (16S qPCR) and diversity (MiSeq Illumina) and to the geographical origins of the air masses from which they originated. Specifically, the ECMWF ERA-5 3D kinematic trajectory model was used to retrace the 72-hours backward trajectories of all the air masses reaching the observatory during each week of sampling.

Our results highlight the presence of 32 different subtypes of airborne ARGs, whose total concentration and related diversity fluctuated seasonally (from 59 to 1.1 x 10^5 copies.m^{-3}), although they were dominated by quinolone resistance overall (> 95%). Such variations were partially related to bacterial abundance and diversity associated with changes in the surrounding landscapes, and with the time spent by the air masses over the continent before reaching the sampling site. Specifically, epiphytic bacteria (*i.e. Sphingomonas)* were particularly abundant during the spring and summer, concomitant with the highest concentrations of ARGs, and could be significantly associated with 9 different resistance genes. Conversely, winter was characterized by a higher contribution of macrolide resistance, which could not be associated to any specific genera. Interestingly, the least abundant genera (<1% of relative abundance) were overall those with the highest number of different resistance genes, exhibiting positive associations with up to 15 different subtypes. Overall, the results of our study indicate that the atmosphere is an important vector of ARGs at short to large spatial scales.

P6

Mechanism reducing carbapenem susceptibility of environmental *E. coli* isolates

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During a 16-month longitudinal study, bi-monthly water samples were taken upstream and downstream of a watershed. In order to detect carbapenem-resistant *E. coli*, the CHROMID®Carba medium was used. Of the 800 isolates collected from 144 samples, *E. coli* identification was confirmed for only seven of them, isolated on the same day, two upstream and five downstream. For six isolates (five downstream and one upstream), a reduced susceptibility to carbapenems was observed (values for ertapenem: diameter of 19 mm and MIC (in microdilution) between 0.5 and 2 $mg.L^{-1}$). The results of the whole genome sequencing indicate that five of the six isolates belong to the same clone (O8:H7, ST196). Furthermore, a mutation of the *ompC* porin coupled with the presence of the bla_{CMY-2} gene, on an *IncI1* plasmid, would be at the origin of the reduced sensitivity of these strains to carbapenems. This type of mechanism has already been described in human clinical cases in the USA, France and Taiwan. To our knowledge, this is the first time that it has been identified in strains from the aquatic environment. The detection of *E. coli* with reduced susceptibility to carbapenems one time in 18 months (one out of 36 sampling dates) could be considered as a one-time event. However, this illustrates the importance of monitoring the aquatic environment but also the methodological difficulties of such surveillance due to the poor performance of the isolation method.

P7

Aerosolization behavior of antimicrobial resistance in animal farms: a field study from feces to fine particulate matter

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Antibiotic resistance genes (ARGs) and antibiotic-resistant bacteria (ARB) in animal feces can be released into the atmosphere via aerosolization, posing a high health risk to farm workers. Thus far, little attention has been paid to the characterization of the aerosolization process. In this study, fecal and fine particulate matter (PM2.5) samples were collected from 20 animal farms involving swine, cattle, layers, and broilers, and the ARGs, ARB, and human pathogenic bacteria (HPB) were loaded in these two media. The results showed that approximately 70% of ARGs, 60% of ARBs, and 43% of HPBs were found to be preferential aerosolization. The bioaerosolization index (BI) of target 30 ARGs varied from 0.04 to 460.07, and the highest value was detected from *tetW*. The highest BI values of Erythromycin- and Tetracycline-resistant bacteria were for *Kocuria* (13119), and *Staphylococcus* (24746), respectively, and the distribution of BI in the two types of dominant ARB was similar. Regarding the bioaerosolization behavior of HPB, *Clostridium saccharolyticum WM1* was the most easily aerosolized pathogen in swine and broiler farms, and *Brucella abortus* strain *CNM 20040339* had the highest value in cattle and layer farms. Notably, the highest BI values for ARGs, ARB, and HPB were universally detected on chicken farms. Most ARGs, ARB, and HPB positively correlated with animal age, stocking density, and breeding area. Temperature and relative humidity have significant effects on the aerosolization behavior of targets, and the effects of these two parameters on the same target are usually opposite. The results of this study provide a basis for a better understanding of the contribution of animal feces to airborne ARGs and HPBs in farms, as well as for controlling the transport of the fecal microbiome to the environment through the aerosolization pathway.

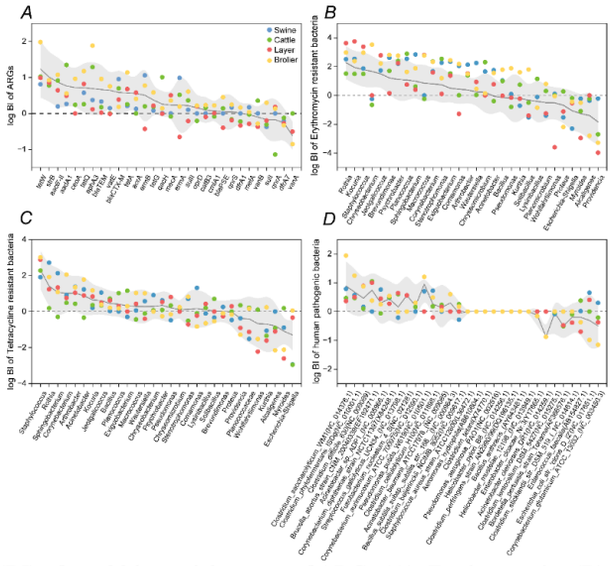

Figure 1 Bioaerosolization Index (BI) of top 30 bacterial genera of ARGs (A), Erythromycin- (B) and Tetracycline-resistant bacterial genera (C), and top 30 human pathogenic bacteria (D). The BI value was log-transformed.

P8

Measuring the effects of antimicrobial residues in the environment on AMR transmission in a duck model.

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Background: In addition to farm animals, wild animals are also often exposed to anthropogenic antibiotic pressure through the contamination of wildlife habitat. Examples include the run-off of antibiotic residuals which are present in manure or the presence antimicrobial residues in effluent water from waste-water treatment plants. As such, various examples of concerning presence of resistant bacteria in wild animals have been described.[1] Transmission of resistant bacteria between aquatic birds through the environment was previously shown in a wild mallard model.[2]
Objective: To determine the effects of antimicrobial residues in pond water on the transmission of resistant *E. coli* in a duck model.
Methods: Nine groups of 15 peking ducks were housed for 2 weeks in enclosures containing a pond with various concentrations of either enrofloxacin or ceftiofur, which was refreshed daily. Animals were inoculated with isogenic *E. coli* strains, with or without an IncI1 plasmid, containing either a *qnr*S1 or a $bla_{\text{CTX-M-1}}$ gene. Samples were collected from individual animals and cultured to enumerate colonisation by resistant *E. coli* using selective MacConkey agar plates. The effects on the gut microbiota were determined by 16s sequencing.
Results/Discussion: Measurement of the residues of antimicrobials in the pond water and faeces showed that the residues would be present at similar in the gut as in the pond water. A rank-based regression analysis of the semi-quantitative culture results was performed. Both the *qnr*S1 and $bla_{\text{CTX-M-1}}$ genes could be detected in the lowest concentrations of either enrofloxacin or ceftiofur but in the highest concentrations (1000 µg/L) the water in the ponds would cause a selective pressure in the gut for either the *qnr*S1 or $bla_{\text{CTX-M-1}}$ gene to be maintained over time. Despite the levels of antimicrobials in the gut, the microbiome developed similarly over time in all groups of animals.

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P9

Environmental characterization of isolated positive patients for carbapenem-resistant Enterobacteriaceae and evaluation of carriage by workers

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The hospital care environment may contain bacteria carrying various antibiotic-resistance genes (ARGs). Surfaces and bioaerosols may have a role in the environmental dissemination of these genes or can allow the monitoring of their presence in the medical environment. More precisely, Carbapenemase-producing Enterobacteriaceae (CPE), enzymes that can hydrolyze almost all beta-lactam antibiotics, are of great concern. Although their dissemination by direct and indirect contact is well documented, the role of air in its dissemination is little investigated.
This work aims to identify and quantify genes associated with CPE in the environment of isolated positive patients (air and surfaces). Some rooms with no positive patient are also sampled to evaluate the ARGs spreading. Also, as part of the project, the healthcare workers' hands and foreheads are tested to assess their occupational exposure and carriage to multi-resistant bacteria.
The sampling takes place in 3 hospitals in the province of Quebec. Air sampling is done in the rooms using the stationary SASS® 3100 Dry Air Sampler with a flow rate of 300L/min. Moisturized swabs and sponges are used to investigate the presence of CPE on surfaces. Multiple surfaces in the rooms are sampled: floors (inside and outside), faucets, drains, doorframes, and no-touch surfaces. No-touch surfaces are sampled to evaluate the deposition of bioaerosols. The genes associated with CPE, namely *bla-KPC*, *bla-OXA-48,* and *bla-NDM* in the various samples, were quantified by quantitative PCR (qPCR), using specific probes and primers. Total bacteria were also quantified using qPCR targeting 16S ribosomal RNA gene.
Rooms from *bla-KPC*-positive patients have been sampled. Preliminary data revealed that the *bla-KPC* gene was found on the doorframe in 50% of cases, and it was also found on the patient room floors in 66% of cases and on the floor outside in 83%. *The bla-KPC* gene was also found on no-touch surfaces but less frequently. Surprisingly, *bla-KPC* gene was detected from the sample in one negative patient's room floor. However, no *bla-KPC* has yet been detected in the air of the positive patients' rooms. Analysis and sampling are still ongoing, including workers' sampling. More sampling in hospitals and workers is planned in the next months and another hospital was recruited to the project, to ensure a better statistical power. Bacterial cultures of a few samples will be performed to know if bacteria carrying ARGs are viable. Results achieved in this study will help characterized ARGs in the hospital environment but also provide a better understanding of the role of air in the spread of multi-resistant and thus make the work environment safer.

P10

Characterization of antibiotic resistance genes in the air of the health care environment

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Various classes of antibiotics are used in human health to treat numerous infectious diseases. The ability to resist antibiotics is conferred to bacteria through antibiotic-resistant genes (ARGs) that can be acquired and/or transferred by bacteria through different mechanisms. The indoor medical environment could represent a potential reservoir for different ARG classes that can potentially be dispersed. The presence of ARGs in the air and the role of the bioaerosols in their propagation have not been extensively studied. This transmission route should be investigated to describe and monitor the antibioresistance evolution in the indoor medical environment.

This work aims to describe the presence of various classes of antibiotic resistance genes (ARGs) and mobile genetic elements in the indoor air of different hospital public areas (cafeteria, corridors, admission, offices). Air sampling was achieved using stationary high-flow air samplers coupled to filters (SASS® 3100 Dry Air Sampler) at different flow rates (200L/min and 300L/min). Each area was sampled three times. Total bacteria concentration in the air was quantified using quantitative PCR (qPCR) targeting the 16S ribosomal RNA gene. The quantification of 34 different subtypes of ARGs from different classes will be achieved by qPCR using specific assays based on a global study focusing on airborne ARG monitoring in various environments (George et al., 2022).

Preliminary data reveal that several beta-lactam resistance genes, such as *blaTEM* and *blaCTXM-1* were detected and quantified in the air of all 4 areas in a hospital. *Bla-KPC, bla-CMY2* and *bla-VEB* were also detected in this same hospital. We expected to find similar results as part of a sampling campaign in another hospital in the province of Quebec.

Results will help assess the presence of ARGs in the air of health care environment and provide a better understanding of the role of the aerosol transmission route in the spread of ARGs to general settings. The use of air samples in the environmental investigation could be used to monitor the presence of ARGs.

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P11

Monitoring antibiotic resistance in a water environment with a minor degree of anthropization: The case of the KAMECH watershed

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Antibiotic resistance remains a major global concern. Indeed, the anthropization of medicines has created a selection pressure favorable to the development of resistant bacteria. The spread of antibiotic resistance in the environment is due not only to the massive and uncontrolled use of antibiotics, but also to their therapeutic and prophylactic use on livestock farms, in agriculture and aquaculture. The aim of this study is to investigate the potential presence of multidrug resistant (MDR) bacteria in a water ecosystem with limited anthropization (deep waters) in the KAMECH catchment area. Our sampling included 9 separate points: 7 wells, a drinking water source and KAMECH lake. Among the one hundred deep-water samples analyzed, 52 bacteria were isolated, of which 37 non-enterobacterial Gram negative bacilli are β-lactam-resistant, 9 are producers of metallo-β-lactamase (MBL) and 2 strains are extended-spectrum- β-lactamase (ESBL) producers. Genotypic analysis revealed the presence of the *bla*$_{NDM}$ gene in 5 strains (*Burkholderia cepacia* and *Pseudomonas oryzihabitans*) and the presence of the *bla*$_{CTX-M15}$ gene in 2 strains (*Stenotrophomonas maltophilia* and *Klebsiella pneumoniae*). The dissemination of these MDR bacteria through water represents a major risk, not only for human health, but also for animal and environmental health.

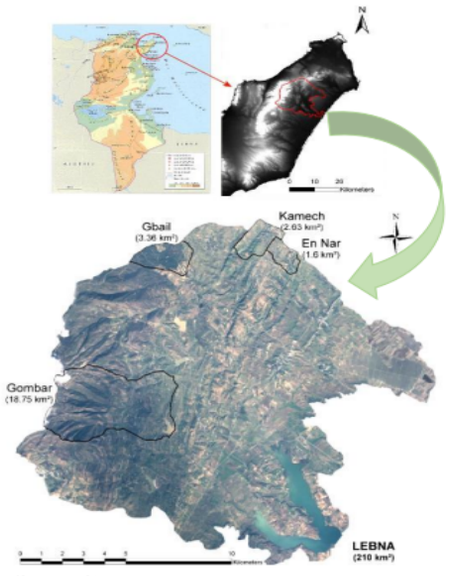

Fig. 1. Geographical location of sampling area

P12

Could *Mytilus galloprovincialis* mussel be a hot-spot of horizontal resistance gene transfer in acquatic environment?

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Antimicrobial resistance (AMR) is one of the most global concerns that recently poses a threat to public health. The continual release of antibiotic resistant bacteria into the environment and their persistence suggests the role that environment may play as an important reservoir and dissemination route of antibiotic resistance genes (ARGs). Horizontal gene transfer (HGT) allows the exchange of genetic elements between different strains and species leading to bacterial genome evolution and contributing to the rapid spread of AMR. Aquatic ecosystems have also become hot-spot for horizontal gene transfer of ARGs representing niches where resistance evolution can occur[1]. Several studies have reported the presence of multidrug-resistant bacteria in coastal waters that could be the result of the exchange of genetic material, such as plasmids, between marine and contaminating bacteria[2]. Bacterial population density and cell proximity differentially impact the various mechanisms of HGT[3]. For this reason, in this study, we investigated the potential of marine bivalve molluscs to become an optimal hot-spot where conjugation can occurs exploiting their ability to concentrate microorganisms from surrounding water as a result of their filter-feeding activity. To this aim, bivalves were cultured in aquaria in which they were exposed to a mating pair of linezolid resistant enterococci and periodically checked for the development of transconjugants. First, the bioaccumulation capacity of the mussels and the ability of the strains to survive in water were evaluated. Bacterial bioaccumulation experiments showed that the greatest values within the mussels were reached within 72 hours from inoculation. Therefore, conjugation experiments were performed with a maximum duration of 72h, inoculating donor and recipient simultaneously and in a 1:1 ratio. All the transconjugants were obtained within 24h. Conjugation experiments conducted at different temperatures showed that the highest conjugation frequency ($\sim 10^{-7}$) was obtained at 20°C temperature, while no conjugation occurs at temperature below 20°C. Transconjugants were found in the various body districts of the mussel at similar frequencies ($\sim 10^{-7}$) both in the digestive gland and in haemolymph. No conjugation seemed to have occurred in the gills. In this study we hypothesized that mussels provide a suitable matrix that might facilitate genetic exchange between pathogenic resistant bacteria reaching coastal waters through sewage. Mussels could contribute both to the spread of ARGs in aquatic environments and to the return of resistant bacteria to human via their consumption.

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P13

How *Salmonella enterica* acquires resistance to Basil's essential oils, and how this affects the bacteria's characteristics

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Essential oils (EOs) are complex mixtures of volatile compounds produced in various parts of plants, and are widely used as natural preservatives in food, beverages, fragrances, sanitary, cosmetics, and pharmaceutical markets. Linalool, one of the main components of the basil's essential oil (BEO), has antibacterial activity. It perforates the bacteria's outer membrane, inhibits cell motility and forms bacterial aggregates. In 2007 a foodborne outbreak originating from contamination of prepacked fresh basil spread across Europe. It was caused by Linalool resistant *Salmonella* Senftenberg (*S.* Senftenberg). It was also demonstrated that the development of resistance to linalool is associated with cross resistance to several antibiotics[1]. In the current study, we hypothesize that extensive commercial use of EOs can lead to the development of resistant pathogens, similar to the acquisition of multiple antibiotic resistance caused by the overuse of antibiotics. In order to understand the specific evolutionary path of acquiring resistance to basil oil and its main components we sequentially exposed *S.* Senftenberg and *S.* Typhimurium to increasing concentrations of linalool under different conditions. In total, we developed 16 different lineages of *S.* Senftenberg. Isolates from these lineages could grow with up to 16% linalool (MIC increased by up to 128 folds in comparison with the WT strain). For *S.* Typhimurium we developed 8 different lineages, but the bacteria were not able to grow with more than 2% linalool (MIC increased by up to 32 folds in comparison with the WT strain). Furthermore, we were interested to study a potential link between basil oil resistance and multiple antibiotic resistance in food borne pathogens. To do so we selected linalool resistant variants from the above described lineages and tested their susceptibility to antibiotics (according to CLSI guidelines). We observed various phenotypic differences out of 15 selected variants, 8 were resistant to trimethoprim, and 1 to kanamycin. On the other hand, 7 variants were found to be susceptible to nalidixic acid. Whole genome sequencing of 50 *S.* Senftenberg variants revealed a total of 66 Single nucleated polymorphisms (SNPs), 52 of them resulted in a single amino acid change. In the *S.* Typhimurium lineages (total of 17 variants were sequenced), 5 SNPs were found, from which 1 resulted in a single amino acid change and 4 resulted in a gap, leading to a truncated protein. The SNPs found were located in different genes, some are responsible for multidrug resistance (*ramA*), heat shock response (*hsUI*), oxidative stress response (*rcsC*) and virulence (*lon*). Moreover, stable linalool resistant variants were developed with no genetic variations. These results indicate that the genetic changes occurring after sequential exposure to increasing concentrations of linalool are random. Further research is required to fully understand how the resistance to linalool accompanied by the change in antibiotic susceptibility occurs, and what precautions should be recommended in order to prevent or control the development of EOs resistant pathogens.

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P14

Uncovering the Hidden World of Caves Reveals the Key Role of Multidrug Efflux Pumps in Formation of Resistomes

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Antibiotic resistance, a natural phenomenon, has become a global concern due to the misuse of antibiotics. Human activities have a substantial impact on the environment, which serves as a reservoir for antibiotic resistance genes (ARGs). Exploring pristine environments free from human interferences provides unique insights into the natural development of microbiomes and resistomes, the collection of all ARGs within a community. The current studies examined 47 publicly available metagenomes from 14 caves, classified into five microhabitats: sediments, microbial mats, water, biofilms, and minerals. Our findings revealed a core microbiome across all samples, with specific taxa displaying differential abundance and discrimination in various microhabitats due to their distinct environmental conditions. Moreover, microhabitats led to significantly different bacterial communities and resistomes. Notably, multidrug resistance (MDR) genes, primarily multidrug efflux pumps, were prevalent in all samples. The resistome differences mainly originated from variations in multidrug efflux pumps, such as *mex*-, *emr*-, and *mdt*-type efflux pumps. Interestingly, the mobile colistin resistance gene *mcr* was pervasive in cave environments. Furthermore, bacterial hosts carrying MDR genes, particularly multidrug efflux pumps, maintained a high relative abundance in ARG-host profiles. These results align with our previous studies using a germfree soil model, which demonstrated that the hosts for MDR genes, such as Burkholderiaceae, are crucial for the establishment of soil resistomes, irrespective of antibiotic selections[1]. This research provides a comprehensive overview of microbiomes and resistomes in pristine cave habitats. The findings highlight the importance of MDR genes, especially multidrug efflux pumps, in the formation of resistomes and the ancient origin of clinically significant ARG *mcr* and possibly other functions of ARGs that have no clinical values.

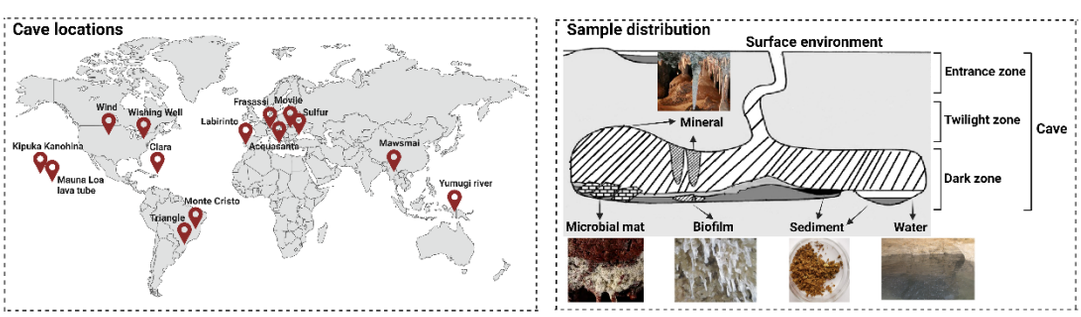

Fig. 1. The locations and types of pristine cave samples

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P15

ICEs in *Vibrio cholerae* from water samples from France: Occurrence, diversity and impact for human health

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Vibrio cholerae is found in aquatic ecosystems throughout the world. While isolates of the O1/O139 serogroup are capable of causing cholera due to the presence of cholera toxin (CTX) and toxin coregulated pilus (TCP) as primary virulence factors, isolates of other serogroups (non-O1/O139) are usually considered less harmful. However, some of them have been shown to be involved in intestinal and extra-intestinal infections (i.e., wound, soft tissue and ear infections or bacteraemia) due to a wide range of virulence factors (i.e., mannose-sensitive haemagglutinin pilus (MSHA), various hemolysins, repeat toxin clusters (RTX), outer membrane proteins and the type III secretion system (TTSS)) that usually act synergistically during the infection process. Besides pathogenicity, self-transferring integrative conjugative elements (ICEs) gain further importance as they represent large genomic segments carrying several bacterial adaptive functions including antimicrobial resistance (AMR). In this study the occurrence and diversity of ICE-carrying *V. cholerae* from France and their impact for human health was studied.

V. cholerae isolation was conducted from water/sediment samples (total: n=586) collected along a salinity gradient under a temperate climate (France): freshwater (2 sites: n=49 and n=91), brackish water (n=207) and sediments (n=41) from brackish areas, seawater (n=59) and wastewater (n=139) between June 2000 and September 2001. In addition, 49 samples of cockles were also investigated. Isolates from different sources were subjected to a molecular survey on the presence of ICE. ICE-carrying isolates (n=64) were further subjected to paired-end, short read whole-genome sequencing (WGS) for in depth characterization.

Non-O1/O139 isolates were successfully recovered by cultivation from all sources investigated in this study. Molecular detection on ICE revealed substantial differences between the different water types, while samples from brackish and seawater exhibited the highest number of ICE-carrying *V. cholerae* in comparison to freshwater samples. Bioinformatic analysis revealed 21 different MLST allele compositions among 64 *V. cholerae* isolates, of which 11 belong to unassigned sequence types (ST). The AMR genes detected were linked to chromosomal contigs and conferred resistance to carbapenems (*varG*), colistin (*almG*) and phenicol (*catB*9). The number of virulence factors among the isolates ranged from 151 to 166 and were involved in adherence, biofilm formation, effector delivery systems, exoenzymes, exotoxins, immune modulation, motility, and nutritional/metabolic factors.

The detection of ICE-carrying isolates in about 10% of the analyzed *V. cholerae* provides a first insight into the presence of self-transmissible sequence regions in naturally occurring isolates. The genomes of the isolates found so far are not heavily loaded with AMR, but may represent a hot spot for the acquisition of insertional elements carrying medically important resistance genes. Selective pressures on the bacteria, such as exposure to antimicrobials or residues in water (wastewater), may force their spread and adaptation, but will also hamper medical treatment purposes against *V. cholerae* infections.

Further surveillance and characterization data is needed and will provide information about diversity of ICE in *V. cholerae* and their specific mechanisms for acquiring and spreading AMR.

P16

Microplastics as cargo for pathogenic bacteria and hotspot of antimicrobial resistance genes transfer? Insights into microbial communities and the emergence of antibiotic resistance

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Microplastics (MP) have emerged as a significant source of pollution in marine ecosystems[1]. Microbial biofilms rapidly establish on the surface of these MPs and studying these microcosms is crucial to characterize the role of MP as vector for pathogens, and their role as reservoir of antimicrobial resistance (AMR) genes.

To tackle these questions, we used the fish-pathogen *Vibrio anguillarum*, as model pathogenic bacteria and evaluated by flow cytometry and microscopy the intraspecific variability in the efficiency of colonization of different types of MP: polystyrene spheres (30 µm), and polypropylene and polyethylene terephthalate fragments (38-50 µm). The set of sixteen strains tested revealed a broad spectrum of attachment phenotypes. We therefore looked for an association between colonization phenotype and gene content and phylogenetic relatedness.

Furthermore, we investigated the potential role of MP in structuring microbial community composition in marine environments. MP samples were incubated at three distinct locations in the Mediterranean Sea, representing varying levels of human activities: an aquaculture area (étang de Thau), a harbor (Carnon city), and a less polluted area (national reserve of Cerbères-Banyuls). Variation in composition of the MP-associated biofilms among locations and MP polymers were analyzed by shotgun metagenomics, using MetaSpades[2]. Obtained sequences were screened against the CARD database[3] for AMR gene identification.

To understand the influence of marine MP-associated biofilms on the emergence of antibiotic-resistant pathogenic bacteria, we investigated the impact of natural marine microbial communities on the attachment of three Vibrio strains covering the range of attachment phenotype. Attachment efficiency was measured by comparing attachment to sterile MP versus colonized MP. Additionally, we are planning to study whether the spatial structure provided by MP in the water column promotes horizontal transfer of resistance genes detected in these communities.

It is crucial to decode the potential consequences of MP-associated biofilms on the emergence of antibiotic-resistant pathogenic bacteria. The colonization of microplastics by bacteria in marine environment enables their long-distance transport, facilitating the spread to susceptible new host populations.

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P17

Fluoroquinolone resistance in *E. coli* in the broiler production chain: the role of parent stock and the hatchery

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Objective

Although restrictions on the use of fluoroquinolones in livestock have been imposed in Belgium since 2016, high levels of fluoroquinolone (FQ) resistance are still found in commensal *Escherichia coli (E. coli)* in broilers. The purpose of this study was to describe the spread of FQ resistant *E. coli* throughout the broiler production chain from parent stock farms up to day-old broiler chicks and to investigate their role in the dynamics of the spread.

Materials and methods

In this study, 4 broiler parent stock farms were included (which were supplying eggs to 3 different hatcheries). On these parent stock farms, 30 faeces samples, 30 eggs, swabs of the egg trays and information on antimicrobial usage, were collected. The batch of on-farm sampled eggs of each parent stock farm was traced to the hatchery and again 30 eggs of the same batch were sampled on day 18 of hatching. On the day of hatching, 30 day-old chicks were collected from the same batch as well. Environmental swabs were collected in the hatchery from several crucial points where the eggs or day-old chickens came into contact with the environment, being the hatching crates, suction cups, collection belt, and the transport crates of the day-old chicks. Both the outside of the eggs, the crushed eggshell, and the contents of the eggs were analysed following the method of Mehzoud *et al.* (2016) [1]. In all samples, the presence of *E. coli* and FQ resistant *E. coli* isolates was detected by plating on plain MacConkey agar or supplemented with 0.25 µg/ml enrofloxacin (ECOFF). All isolates were identified using MALDI-TOF MS. For a selection of isolates whole genome sequencing was applied to investigate their phylogenetic relatedness using cgMLST analysis, and to detect resistance determinants.

Results

On all 4 parent stock farms, FQ resistant *E. coli* were detected [56.7-93.3%]. A low number of eggs was positive for *E. coli,* both just after laying [0-13.3%] and after 18 days of incubation [0-3.3%]. Except for one egg after laying, no FQ resistant *E. coli* could be found in or on the eggs. In the day-old chicks sampled at the hatchery, 3 out of 4 batches were positive for FQ resistant *E. coli* [0-90%]. On the day of hatching all environmental swabs from the collection belts were positive for FQ resistant *E. coli* and in 3 out of 4 batches, FQ resistant *E. coli* was discovered on the transport crates of the 1-day-old chicks. The results of the whole genome sequencing will be presented during the conference.

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P18

Multi-drug resistant *Klebsiella* spp. in hospitals, wastewaters and surface waters

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Multi-drug resistant bacteria pose a significant challenge to the treatment of infectious diseases. Of particular concern are bacteria belonging to the genus *Klebsiella*, especially *K. pneumoniae* (*Klpn*) and *K. oxytoca* (*Klox*), which are frequently associated with hospital-acquired infections and have potential to spread outside hospitals via wastewaters. The municipal wastewater treatment plants (mWWTP) have been identified as a reservoir of resistant bacteria and are considered a hotspot that facilitates the spread of antibiotic resistance into the environment. The aim of this study was to compare clinical isolates of *Klebsiella* spp. with resistant strains obtained from wastewaters and surface water.

A total of 397 *Klebsiella* spp. (383 *Klpn* and 14 *Klox*) were obtained from patients (n=138) and five types of water: hospital sewage (n=97), inflow (n=58) and outflow from the mWWTP (n=73), river upstream (n=22) and downstream mWWTP (n=19) from three cities in Czech Republicusing selective cultivation (cefotaxime, 2 mg/l). All strains were tested for minimum inhibitory concentrations of 24 antimicrobials, extended-spectrum beta-lactamase (ESBL) & AmpC production and subjected to whole-genome sequencing (Illumina). Analysis of antibioticresistance genes, sequence types (STs) and plasmid replicons was performed by BLAST algorithm and CGE tools (http://www.genomicepidemiology.org/). The phylogenetic tree was constructed using Prokka, Pirate and RAxML.

Most isolates (95%) showed multi-drug resistance profile, resistance to cefazolin (100%), aminoglycosides (92%), trimethoprim (87%), ciprofloxacin (76%), tetracyclines (64%) and some of them were resistant to last line drugs carbapenems (14%) and colistin (8%). Resistance to cephalosporins was associated with ESBL phenotype and encoded mainly by $bla_{\text{CTX-M-15}}$ gene (n=379/397). Carbapenemase-encoding genes including $bla_{\text{GES-5}}$ (n=15), $bla_{\text{OXA-48}}$ (n=4), $bla_{\text{NDM-1}}$ (n=4) and $bla_{\text{KPC-3}}$ (n=1) were found in isolates originating from patients, hospital sewage, outflow/inflow of mWWTPs and river. Isolates were assigned to 81 different STs with predominance of *Klpn* ST307 (n=53) that was found in all water types and clinical samples. Among *Klox*, ST27 (n=7) was the most prevalent, found in outflow from mWWTP and river upstream mWWTP and showed high phylogenetic relatedness (SNPs<50).

This study pointed out the importance of the surveillance of pathogenic resistant bacteria in the hospital and community wastewaters and their spread through the WWTPs to surface water and the environment.

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P19

Characterisation of extended spectrum cephalosporin resistance in *Escherichia coli* on Irish pig farms using whole genome sequencing

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Extended spectrum cephalosporin (ESC) resistance in *Escherichia coli* is a major public health issue. Although not the primary source of human infections, animals represent an important reservoir of extended spectrum (ESBL) and AmpC beta lactamase producing *E. coli*. As one of the highest users of veterinary antimicrobials, the pig sector is a particular focus of concern and there is a need for further studies investigating the epidemiology and genetic context of ESC-resistant *E. coli* during the pig lifecycle. This study sought to investigate the prevalence and molecular epidemiology of ESBL- and AmpC producing *E. coli* in batches of pigs on Irish farms during their lifecycle.

Twelve farrow-to-finish farms participated in a longitudinal study. Ten litters of piglets were selected from each farm; pooled faecal samples were collected during each production stage (piglet, weaner, grower and finisher), at slaughter, from the piglets' dams and also from unrelated batches in different houses. ESC-resistant *E. coli* were selected using media supplemented with 1 mg/L cefotaxime and plate counts were used to calculate the proportions of resistance. Up to five isolates from each sampling point were selected for antimicrobial susceptibility testing (AST) and whole genome sequencing (WGS).

All farms were positive for ESC resistant *E. coli* at at least one sampling point during the study. The highest proportions of resistance were observed in piglets (median = 0.00005, range = 0 - 0.15) and were lowest in finishers (median = 0, range = 0 - 0.01). In total, 198 isolates were selected for AST and WGS analysis. In addition to beta lactam and cephalosporin resistance, 95% of isolates were resistant to at least one other antimicrobial from different classes such as: doxycycline (82.3%); trimethoprim and sulfamethoxazole (64.6%); gentamicin (21.7%) and ciprofloxacin (67.2%). Eighty-seven isolates were positive for an ESBL gene, mainly $bla_{\text{CTX-M-15}}$ (n = 57), $bla_{\text{CTX-M-27}}$ (n = 18), $bla_{\text{CTX-M-65}}$ (n = 10). The other isolates were AmpC producers and possessed either $bla_{\text{CMY-2}}$ (n = 46) or a mutation of the chromosomal *amp*C promoter gene (n = 64). Twenty-six sequence types (ST) and 26 serotypes were identified with 40 unique combinations. Each unique combination of ST and serotype was associated with one ESC resistance mechanism. One ESC resistance mechanism dominated on each farm throughout the production cycle although six farms were positive for more than one ESBL/AmpC producing gene. In addition, $bla_{\text{OXA-1}}$ was found in 33 isolates on three farms and in all cases co-occurred with the dual aminoglycoside and fluoroquinolone resistance gene, *aac*(6')-Ib-cr.

This study gives important insights into the epidemiology and diversity of ESC resistant *E. coli* on Irish pig farms. More in depth bioinformatical analysis of this data is to follow and will allow for determination of phylogeny and the zoonotic or pathogenic potential of the isolates and for improved genetic context of the detected resistance genes.

P20

Hospital and community wastewaters as a source of multidrug-resistant ESBL-producing *Escherichia coli*

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Hospitals and wastewaters represent a recognized hot spot for the selection and dissemination of antibiotic-resistant bacteria to the environment, but the total participation of hospitals in the spread of nosocomial pathogens to the municipal wastewater treatment plants (WWTPs) and adjacent rivers were to be revealed. Therefore, we used a combination of culturing and whole-genome sequencing to explore the transmission routes of *Escherichia coli* from hospitalized patients suffering from urinary tract infections (UTI) via wastewaters to the environment. Samples were collected in two periods in three locations (A, B, C) and cultured on selective antibiotic-enhanced plates. In total, 409 *E. coli* isolates were obtained from patients with UTI (n=80), raw hospital sewage (n=74), WWTPs inflow (n=95)/outflow (n=106), and river upstream (n=23)/downstream (n=31) of WWTPs. The majority (96%) of the isolates produced extended-spectrum beta-lactamase, mainly CTX-M-15, and showed multidrug resistance (MDR) profiles. Seven carbapenemase-producing isolates with GES-5 or OXA-244 were obtained in two locations from wastewaters and river samples. Isolates were assigned to 76 different sequence types (ST) with the predominance of ST131 (n=80) that was found in all sources including rivers. Extraintestinal pathogenic lineages frequently found in hospital sewage (ST10, ST38, ST69) were also found in river water. Despite generally high genetic diversity, phylogenetic analysis of ST10, ST295 and ST744 showed highly related isolates (SNP 0-18) from different sources, providing the evidence for the transmission of resistant strains through WWTPs to surface waters. Results of this study suggest that 1/ UTI share a minor participation in hospitals wastewaters; 2/ high diversity of STs and phylogenetic groups in municipal wastewaters stress the urban influence rather than hospitals; 3/ pathogenic lineages and bacteria with emerging resistance genotypes associated with hospitals spread into surface waters. Our study highlights the contribution of wastewaters to the transmission of ESBL- and carbapenemase-producing *E. coli* with MDR profiles to the environment.

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P21

Genotypic characterization of ESBL/AmpC-producing *E. coli* from the German National Zoonoses Monitoring from 2016-2021 within the food production chain

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Extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases are able to hydrolyze 3rd generation cephalosporins, which are widely used in human medicine. Resistances mediated by ESBL/AmpCs are of great concern and closely monitored in human as well as in veterinary medicine. Here, we present data on the ESBL/AmpC genotypes of 4601 *E. coli* isolates obtained from the regular monitoring on antimicrobial resistance (AMR) in zoonotic and commensal bacteria from 2016-2021.

The AMR monitoring was conducted within the framework of the Zoonoses Directive 2003/99/EC, following technical specifications given by Commission Implementing Decision (CID) 2013/652/EU and CID 2020/1729/EU complemented by additional national zoonoses programs. Isolates were obtained from different matrices from broilers (N = 1297), fattening turkeys (N = 1005), fattening pigs (N = 992) and cattle (N = 1206), and at different levels of the food production chain including primary production, slaughterhouse and retail. In addition, isolates from wild animals, small ruminants, freshwater fish and plant-based food were included (N = 101). In the years 2016-2020 phenotypic presumed ESBL/AmpC isolates were analyzed by real-time PCR and genotype was determined by PCR sequencing. TEM variant was only determined if no other possible ESBL gene was detected, as high number of isolates harbor additional $bla_{\text{TEM-1}}$ (non-ESBL). In 2021, presumed ESBL/AmpC-producing isolates were characterized by Illumina short read sequencing.

The variability of ESBLs genes is fairly low with 23±3, different detected genes and gene combinations each year. Although PCR primers used do not allow to detect all known gene variants, variability of ESBL genes found did not increase by NGS analysis. However, NGS supports detection of chromosomal AmpC gene alterations. In general, the majority of isolates harbored one ESBL/AmpC gene, only few carried two or more genes. In cattle and pigs (uneven years), the majority of isolates (75.8 %) produce CTX-M-1 or CTX-M-15, whereas in poultry (even years), a more even distribution of CTX-M-1, CTX-M-15, CMY-2, TEM-52 and SHV-12 was observed. ESBL/AmpC-beta lactamases CTX-M-2, -3,-8, -9, DHA-1 or EC-5 were found rarely. Comparing the genes found at slaughter (caecum content) over the years, there were no major variations in isolates from pigs and calves. However, in broilers a significant decrease in CMY-2 and CTX-M-1 beta-lactamases and a significant increase in SHV-12 and TEM-52 were observed. Significant changes in several genes were also observed in turkeys over time (decrease in CTX-M-1 and TEM-52, and increase in CTX-M-15, -27 and -55). Distribution of ESBL/AmpC genes in isolates from wild animals, small ruminants, freshwater fish and plant-based food was similar to the combined frequency in pigs and cattle.

In conclusion, close observation to detect trends on the specific ESBL genes in different livestock production chains is important to assess the potential sources of human exposure. In addition, further research is needed to understand what caused the shift in poultry, as the use of cephalosporins is not allowed in poultry production

P22

Extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing *Escherichia coli* among bivalve samples of the Portuguese coast

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Background. Bivalves are filter-feeding organisms and biomarkers of bacterial pollution. Our study aimed to analyze the occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) and carbapenemase-producing *Escherichia coli* among bivalve samples from Portuguese coastal waters.

Methods. As part of the national monitoring program for shellfish production area management, 551 bivalve samples were collected between december 2021 and september 2022, by the Instituto Português do Mar e da Atmosfera (IPMA), from 20 shellfish production areas along the Portuguese coastal waters. Homogenized samples were screened for *E. coli* contamination by plating onto tryptone bile X-glucuronide (TBX) selective plates. A total of 206 TBX plates showing *E. coli* bacterial growth (37%) were provided by IPMA for this study. After overnight broth enrichment, the bacterial growth of each TBX plate was inoculated onto selective media for ESBL producers (CHROMagar ESBL) and for carbapenem-resistant isolates (ChromID Carba Smart). Antimicrobial susceptibility testing was performed on all isolates recovered from the two selective media using the disc diffusion method. Screening for ESBL and carbapenemases was performed by PCR amplification and sequencing. Clonality was evaluated by multilocus sequence typing.

Results. Nine bivalve samples were contaminated with an ESBL producer (4.4%) and one carried a carbapenemase-producing isolate (0.005%). The 10 isolates belonged to two species: *E. coli* (n=7; 70%) and *K. pneumoniae* (n=3). All ESBLs were from the CTX-M-type: CTX-M-32 (n=4), CTX-M-15 (n=4) and CTX-M-14 (n=1). The single carbapenemase producer harbored bla_{GES-5}. The seven ESBL-producing *E. coli* isolates belonged to five distinct sequence types (ST10, ST23, ST540, ST617, ST746, SLV206, SLV2325), while the two ESBL-producing *K. pneumoniae* isolates belonged to ST834 and ST15. The carbapenemase producer (*K. pneumoniae*) belonged to a new ST. Mating-out assay followed by PCR-based replicon typing revealed that the bla_{GES-5} gene was located on a ColE plasmid.

Conclusions. Bivalves constitute a reservoir of ESBL- and carbapenemase-producing Enterobacteriaceae. We identified the first enterobacterial isolate producing a carbapenemase of GES-type recovered from bivalve samples. This carbapenemase, which has been previously detected in Portugal among humans, animals and now in the marine environment, seems to be widely spread in the country, despite the prevalence in bivalves is still reduced.

P23

Phylogenetic analysis of *Acinetobacter* spp. isolates from raw meat

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The *Acinetobacter* genus includes 76 species. Among those, *Acinetobacter baumannii* is the most threatening for public health, because of its ability to develop multidrug resistance and to cause opportunistic and severe infections. Multidrug-resistant *A. baumannii*, especially those belonging to international clonal complexes (ICs) I-III, are able to spread epidemically. More recently, the medical importance of *Acinetobacter nosocomialis* has been demonstrated [1]. Furthermore, extra-hospital reservoirs of *Acinetobacter* species have been unveiled with increasing interest for food sources.
The aim of this study was to characterize 76 *Acinetobacter* spp. isolates collected in the frame of two screening campaigns (November 2012-May 2013, December 2018-July 2019) in raw meat samples [2,3]. Susceptibility to 14 antibiotics was evaluated by disc diffusion (EUCAST, 2022). Total DNA was extracted and sequenced generating short-reads (Illumina). After reads' quality check (FastQC v. 0.11.9), assembly was obtained using Shovill v.1.0.4. Identification was confirmed by rMLST (https://pubmlst.org/species-id). Presence of antibiotic resistance genes and determination of the sequence type was achieved using the Comprehensive Antibiotic Resistance Database and the Pasteur Institute scheme, respectively. Genomic relationships among isolates was analyzed by parsnp v.1.5.2.
Two out of 76 isolates were identified as *A. nosocomialis*, the remaining were *A. baumannii* harboring $bla_{\text{OXA-51-type}}$ and $bla_{\text{ADC-25}}$ genes. Thirteen *A. baumannii* isolates were resistant to tetracycline and harbored a *tet*(39) gene. Two possessed the *aph(3'')-Ib* and *aph(6)-Id* genes conferring resistance to streptomycin. The phylogenetic tree representation evidenced heterogeneity among genomes of the study, reflected by the diversity of their sequence types (n=51). Four sequence types (ST42, ST109, ST351, ST364) were present in both screening campaigns and several non-epidemic (n=20) were shared with isolates associated to infections in humans and from diverse regions of the globe.
Raw meat could serve as important reservoir of different clones of *A. baumannii* with potential for causing infections. Through food, such clones could propagate in the community and under selective pressure develop multidrug resistance. Food products need careful surveillance for understanding their role in the global picture of the antibiotic resistance burden.

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P24

Time-calibrated WGS-based phylogeny revealed successful spread of two major Staphylococcus aureus CC398 phylogroups around Europe and novel CC398 MRSA lineages in Switzerland.

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A time-calibrated whole genome-based phylogeny of 130 Swiss methicillin-resistant *Staphylococcus aureus* (MRSA) of clonal complex (CC) 398 originating from animal, food samples, and humans as well as of an international CC398 collection of 638 strains from all 5 continents revealed the presence of distinct phylogroups in Asia, North and South America, and Europe. The majority of Swiss MRSA CC398 clustered within two major European phylogroups (EP1 and EP2) and formed three major novel lineages (LE), LEd in EP1 and LEa, LEb in EP2. The Bayesian inferred phylogeny estimated that the European phylogroups EP1 and EP2 started to diverge from each other in the 1970s. LEa (n=57) is characterized by MRSA CC398 carriage of the staphylococcal cassette chromosome *mec* (SCC*mec*) elements IVa and *spa* type t011 (t011-SCC*mec*IVa), LEb (n=25) by t011-SCC*mec*Vc, and LEd (n=32) by t011/t034/t571-SCC*mec*Vc.

Antibiotic resistance gene patterns of Swiss lineages were conserved within strains of LEa [*aac(6')-Ie – aph(2'')-Ia*, *tet*(M), *dfrK*] (horses, human carriage and infections) and LEd [*ant(9)-Ia*, *tet*(M), *tet*(K), *erm*(A), *vga*(E), *dfrG*] (pigs and cattle carriage), and diverse in LEb strains (carriage and infection in different animals and humans). Although spillover was frequent between human and animals based on phylogenetic relatedness, virulence profiling revealed that there were no human or animal specific virulence factors and human adaptation (i.e. acquisition of the immune evasion cluster) was scarcely detected in human and horse strains.

By placing international CC398 into a temporal, geographical, and phylogenetic context, we showed that some Swiss MRSA CC398 (n=89) from animals and human origin clustered into novel lineages, which evolved, from two globally spreading European phylogroups and that bacterial exchange occurs between host species, with some of them developing into infections especially in horses and humans. This study provides a baseline for future global WGS-based One-Health studies of the evolution of MRSA CC398 as well as for local outbreak investigations.

P25

Antimicrobial and biocide susceptibility among *Staphylococcus aureus* and *Staphylococcus pseudintermedius* from dogs and cats

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Introduction: Since *Staphylococcus aureus* and *Staphylococcus pseudintermedius* have a zoonotic potential, monitoring of their antimicrobial and biocide resistance is an important issue. The aim of the present study was to investigate the resistance situation of canine and feline *S. aureus* and *S. pseudintermedius* isolates from diagnostic submissions.
Materials and Methods: A total of 114 isolates, including 62 *S. aureus*, 52 *S. pseudintermedius* were investigated for (i) their susceptibility to 27 antimicrobial agents by broth microdilution according to CLSI [1] and the detection of the respective resistance genes. Moreover, the isolates were tested for their susceptibility to four biocides, namely benzalkonium chloride, chlorhexidine, polyhexanide and octenidine by a recently developed biocide susceptibility testing protocol [2].
Results: Penicillin resistance mediated by the *blaZ* gene was the dominant resistance property with 80.65% among *S. aureus* and 86.54% *S. pseudintermedius*. About one quarter of the isolates (*S. aureus* 33.87%; *S. pseudintermedius* 15.38%) proved to be methicillin-resistant and carried the genes *mecA* or *mecC*. Macrolide resistance with 27.2% was second most prevalent and all isolates harbored the resistance genes *erm*(A), *erm*(B), *erm*(C), *erm*(T) or *msr*(A), alone or in combinations. Among all isolates tested fluoroquinolone resistance was detected in 21.1% and tetracycline resistance mediated by *tet*(K) and/or *tet*(M) occurred in 19.3% of the isolates. Resistance to last resort antimicrobial agents for human medicine was only detected in single isolates, if at all. The biocide susceptibility testing showed unimodal minimal inhibitory concentration (MIC) distributions of the four biocides and were very similar for *S. aureus* and *S. pseudintermedius*.
Conclusions: While various antimicrobial resistance properties were determined and a considerable number of the isolates displayed a multiresistance phenotype, the biocide susceptibility testing revealed unimodal distributions not pointing towards resistance development. Further monitoring is necessary to early detect changes in the resistance situation.

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P26

Highly conserved IncK/*bla*$_{CMY-2}$ plasmids in *E. coli* ST38 from broiler production

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Escherichia coli belonging to multilocus sequence type (MLST) 38 have been predominant among extended-spectrum cephalosporin (ESC)-resistant *E. coli* detected in Norwegian broiler production. Previously we have shown that IncK/*bla*$_{CMY-2}$ plasmids from broiler production in different European countries are highly similar, but with some differences with regard to size. We were interested in looking into the IncK/*bla*$_{CMY-2}$ plasmid epidemiology and evolution in ESC-resistant *E. coli* ST38 over a six-year period (2011-2016). Isolates for long-read sequencing were selected based on an ST38 phylogenetic tree [1], picking representatives from different parts of the tree, different levels of the production pyramid and different years to get a representative selection. A total of 10 isolate were sequenced using Oxford Nanopore Technology (ONT, minION). Plasmids were assembled with Unicycler, annotated with Bakta and compared using the gggenomes package in R (Figure 1).
We will include complete IncK/*bla*$_{CMY-2}$ plasmids originating from *E. coli* ST38 from broiler production in other European countries, and thus evaluate diversity and, if possible, evolution of the plasmids in a European perspective.

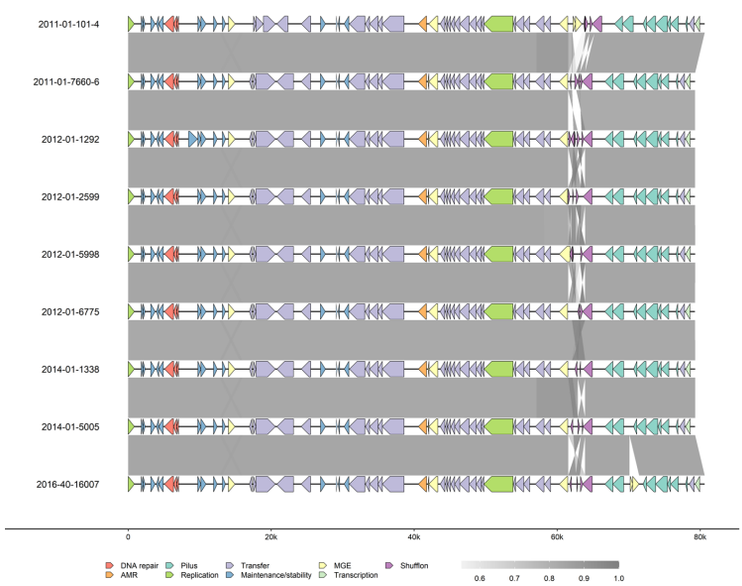

Figure 1. Alignment of IncK/*bla*$_{CMY-2}$ plasmids originating from *E. coli* ST38 isolated from Norwegian broiler production during a six-year period (2011-2016).

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P27

AMR monitoring on rabbit farms

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Background: In 2014 antimicrobial resistance (AMR) monitoring of livestock and meat became mandatory within the European Union (EU). The animal sectors covered by the mandatory monitoring are: veal calves, fattening pigs, turkeys and broilers. However, other animal sectors, such as meat rabbits and dairy goats, are not included. Therefore, no recent AMR data are available from these animal sectors. The latest report of the Netherlands Veterinary Medicines Institute (SDa) shows that antibiotic use in the rabbit sector has decreased but is still relatively high compared to other animal sectors. Therefore, a baseline study on antibiotic resistance in meat rabbit farms in the Netherlands is performed.
Objectives: Monitoring of antimicrobial resistance in the rabbit sector in the Netherlands.
Methods: At each of 15 rabbit farms, five fresh feacal samples were taken from the floor of the pens close to slaughter date. In line with European legislation, indicator *Escherichia coli*, ESBL/AmpC/carbapenemase-suspected *E. coli*, *Campylobacter coli* and *C. jejuni* were isolated from the collected samples. In addition, MRSA was isolated from five dust samples, collected with wipes and up to 5 nose-swabs of dead animals per farm. Antimicrobial susceptibility was tested with broth microdilution method with standard European antibiotic panels.
Results: Bacterial culture was conducted on a total of 75 feacal samples. No ESBL/AmpC /carbapenemase-suspected *E. coli*, *Campylobacter coli* and *jejuni* were isolated from these fecal samples. Also, no MRSA was isolated from dust samples and nose-swabs. Indicator *E. coli* was present in all feacal samples, MIC testing of these isolates showed high percentage of resistance to tetracycline (56.0%), trimethoprim (54.7%) and sulfamethoxazole (52.0%). Five *E. coli* isolates originating from 3 different farms showed a colistin MIC of 4-16 mg/L.

Table 1: MIC-value and resistance percentage (%) of *E. coli* isolated from rabbits

	Antibiotic														
MIC (g/L)	AMP	FOT	TAZ	GEN	TET	SMX	TMP	CIP	NAL	CHL	AZI	COL	MERO	TGC	AMI
0.015	0	0	0	0	0	0	0	36	0	0	0	0	0	0	0
0.03	0	0	0	0	0	0	0	27	0	0	0	0	73	0	0
0.06	0	0	0	0	0	0	0	9	0	0	0	0	2	0	0
0.125	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
0.25	0	71	48	0	0	0	9	0	0	0	0	0	0	50	0
0.5	0	4	23	46	0	0	14	1	0	0	0	0	0	25	0
1	0	0	4	25	0	0	10	1	0	0	0	70	0	0	0
2	2	0	0	4	22	0	1	0	0	0	1	0	0	0	0
4	33	0	0	0	10	0	1	0	71	0	22	0	0	0	72
8	27	0	0	0	1	36	0	0	2	54	45	2	0	0	3
16	0	0	0	0	0	0	0	0	0	17	7	3	0	0	0
32	0	0	0	0	0	0	40	0	0	1	0	0	0	0	0
64	13	0	0	0	42	0	0	0	0	1	0	0	0	0	0
128	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0
256	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
512	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1024	0	0	0	0	0	39	0	0	0	0	0	0	0	0	0
2048	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N tot	75	75	75	75	75	75	75	75	75	75	75	75	75	75	75
R%	17.3%	5.3%	0.0%	0.0%	56.0%	52.0%	54.7%	4.0%	2.7%	5.3%	0.0%	6.7%	0.0%	0.0%	0.0%
Nres	13	4	0	0	42	39	41	3	2	4	0	5	0	0	0

Conclusion and discussion: In indicator *E. coli* from meat rabbits resistance levels are high for tetracycline, trimethoprim and sulfamethoxazole compared to livestock. This might reflect the relatively high antibiotic use in the rabbit sector. In contrast, no ESBL/AmpC/carbapenemase-suspected *E. coli*, *C. coli* and *C. jejuni* were isolated from feacal samples and no MRSA was isolated from dust or nasal swabs.

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P28

Unexpected high resistance rates for ertapenem in *Campylobacter* isolates obtained from livestock

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Background: A new European legislation on antimicrobial resistance monitoring in livestock and meat came into force in 2021 based on a scientific EFSA report [1]. Therefore, susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli* isolates obtained from broilers and cattle (<1 year) and from *C. coli* isolates from fattening pigs were included in the mandatory monitoring program. In addition, the harmonised antimicrobial panel for susceptibility testing of *Campylobacter* was modified: nalidixic acid and streptomycin were substituted by chloramphenicol and ertapenem.
Methods: Isolation of *Campylobacter* was performed according to a standardised protocol designed by the EURL for *Campylobacter* [2]. Susceptibility testing was performed with broth microdilution in standardised European antibiotic panels (Sensititre, panel EUCAMP3) according to ISO 20776-1. Results were interpreted using epidemiological cut-off values (ECOFFs) published by EUCAST. For ertapenem, tentative ECOFFs of 0.5 mg/L (advised by EFSA) were applied for both *C. jejuni* and *C. coli*. As a follow-up, a set of 30 *Campylobacter* isolates with variable ertapenem MIC-values were selected for further analysis conducting Whole Genome Sequencing (Illumina) to reveal the presence of potential carbapenem resistance mechanisms.
Results: In 2021 AMR monitoring of *C. jejuni* and *C. coli* from caecal samples of broilers was mandatory. In addition, AMR monitoring of *C. coli* from fattening pigs and *C. jejuni* and *C. coli* from cattle were included in the Dutch AMR monitoring program on a voluntary base. Applying the tentative ECOFF for ertapenem resulted in high resistance rates for ertapenem, especially in *C. coli* from poultry (76.2%) and to a lesser extent in broilers (39.4%) compared to low resistance in *C. coli* from fattening pigs (2.8%) and *C. jejuni* from broilers and veal calves. WGS data analysis showed the presence of bla_{OXA} genes in 27 out of 30 isolates: $bla_{\text{OXA-184}}$ (n=2), $bla_{\text{OXA-193}}$ (n=15) and $bla_{\text{OXA-489}}$ (n = 9), without association with elevated ertapenem MIC-values.

Table 1. Ertapenem resistance percentages of *C. jejuni* and *C. coli* isolated from faecal samples of livestock in 2021

C. jejuni (R%)			*C. coli* (R%)		
Broilers (n = 131)	Veal calves (n = 222)		Broilers (n = 84)	Veal calves (n = 137)	Pigs (n = 287)
6.1	0.5		76.2	39.4	2.8

Discussion and conclusions: The tentative ECOFFs for ertapenem seems to overestimate the proportion of resistance for ertapenem, especially for *C. coli* by cutting through the wild-type population. Nonetheless, the observed animals-specific differences in ertapenem resistance remains unexplained after analysis of the WGS data. Additional research is required to uncover intrinsic or acquired carbapenem resistance mechanisms in both *C. jejuni* and *C. coli*.

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P29

Resistances to Cephalosporins and Fluoroquinolones in Veterinary medicine in Livestock
Results of the German National Antibiotic Resistance Monitoring

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Objectives

Cephalosporins (3rd and 4th generation) and Fluoroquinolones are classified from WHO as "highest priority critically important antimicrobials" for human use and from WOAH as "critically important antimicrobials". They should be used careful and prudent and only then where it is necessary. Nevertheless, they are important substances to treat bacterial infections in human and veterinary medicine. Since 2001, an annual representative German-wide monitoring study (GE*RM*-Vet) on bacterial isolates from diseased animals generates resistance data amongst others against a set of five different cephalosporins and a set of three different fluoroquinolones.

Methods

Based on a statistically valid sampling plan the bacterial isolates were investigated by using the broth microdilution method according to CLSI document VET01 5th ed. The MIC values were assessed with their corresponding clinical veterinary breakpoints). If no breakpoints were available, MIC_{90} values were used for classification.

Results

The resistance data were evaluated according the bacterial species, animal species and indications that were investigated.

Mastitis (dairy cow)

S. aureus isolates showed low resistance rates against cephalosporins (0-1.5%) and fluoroquinolones (MIC_{90} 0.25 mg/L), *E. coli*: MIC_{90} values were low over a period of five years (Cefquinom MIC_{90} 0.12 mg/L, Ceftiofur about 3% in 2021).

Calves

MIC_{90} values for cephalosporins of the 3rd and 4th generation and for fluoroquinolones are high for bacterial strains isolated from calves (for all tested cephalosporins >32 mg/L, fluoroquinolones >16mg/L). The rate for ESBL positive *E. coli* isolates from calves is decreasing since 2017 from 32% to 13% in 2021. ESBLs of the class $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M}$-15 were identified most frequently

Pigs

MIC_{90} values for cephalosporins of the 3rd and 4th generation and fluoroquinolones were much lower for bacterial strains isolated from pigs than for those isolated from calves (for all tested cephalosporins 0.12 – 0.5 mg/L, fluoroquinolones 1 mg/L). The rate for ESBL positive *E. coli* was still low about 3% in 2021.

Poultry

Cephalosporins are not approved for veterinary use in poultry. Nevertheless, we see high MIC_{90} values for broilers although the ESBL rates for *E. coli* are still at 2%. Resistance rates for isolates from turkeys to fluoroquinolones are low (about 7%) for isolates derived from broilers the values are slightly higher (16%.

Conclusions

An intelligent and rational application of antimicrobial agents is needed to minimise the development and the spread of antimicrobial resistant bacteria and their resistance genes as far as possible. Depending on the affiliation to animal and bacterial species we see large differences in resistance data and a very different impact on resistance situation in veterinary medicine. With these representative and quantitative data, we are able to monitor and to estimate the development of antimicrobial resistance in veterinary pathogens to 3rd and 4th generation cephalosporins.

P30

Linezolid-resistant *Enterococcus faecalis* of chicken origin harbored chromosome-borne *optrA* and plasmid-borne *cfr*, *cfr*(D) and *poxtA2* genes

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The aim of this study was to investigate the transferability of acquired linezolid resistance genes and associated mobile genetic elements in an *Enterococcus faecalis* isolate QZ076, co-carrying *optrA*, *cfr*, *cfr*(D) and *poxtA2* genes. MICs were determined by broth microdilution. WGS was performed using the Illumina and Nanopore platforms. The transfer of linezolid resistance genes was investigated by conjugation, using *E. faecalis* JH2-2 and clinical MRSA 109 as recipients. *E. faecalis* QZ076 harbors four plasmids, designated pQZ076-1 to pQZ076-4, with *optrA* located in the chromosomal DNA. The gene *cfr* was located on a novel pseudo-compound transposon, designated Tn*7515*, integrated into the 65,961-bp pCF10-like pheromone-responsive conjugative plasmid pQZ076-1. Tn*7515* generated 8-bp direct target duplications (5'-GATACGTA-3'). The genes *cfr*(D) and *poxtA2* were co-located on the 16,397-bp mobilizable broad-host-range Inc18 plasmid pQZ076-4. The *cfr*-carrying plasmid pQZ076-1 could transfer from *E. faecalis* QZ076 to *E. faecalis* JH2-2, along with the *cfr*(D)- and *poxtA2*-co-carrying plasmid pQZ076-4, conferring the corresponding resistant phenotype to the recipient. Moreover, pQZ076-4 could also transfer to MRSA 109. To the best of our knowledge, this study presented the first report of four acquired linezolid resistance genes [*optrA*, *cfr*, *cfr*(D), *poxtA2*] being simultaneously present in the same *E. faecalis* isolate. The location of the *cfr* gene on a pseudo-compound transposon in a pheromone-responsive conjugative plasmid will accelerate its rapid dissemination. In addition, the *cfr*-carrying pheromone-responsive conjugative plasmid in *E. faecalis* was also able to mobilize the inter-species transfer of the *cfr*(D)- and *poxtA2*-co-carrying plasmid between enterococci and staphylococci.

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P31

Antimicrobial resistance of *Escherichia coli* isolated from slaughterhouse effluents in France: monitoring 15 years apart

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Introduction. Livestock is a reservoir of antibiotic resistant *E. coli* strains, and such strains are present in slaughterhouse effluents. As the presence of these strains is poorly documented, the aim of this study was to identify factors having an impact on the prevalence of antibiotic-resistant *E. coli* in slaughterhouse effluents: species slaughtered, season, step of sewage treatment. Moreover, some effluents were sampled after an interval of 10/15 years in order to estimate the impact of the French national plans Ecoantibio, implemented in 2012, which aimed to reduce the use of veterinary antibiotics.

Materials and methods. *E. coli* strains were isolated from effluents of various types of domestic ungulate slaughterhouses, at different sampling campaigns:

(i) slaughterhouses with large tons carcass weight equivalent per year, possessing a Wastewater Treatment Plant (WWTP), allowing them to discharge the treated effluents into the environment: 6 slaughterhouses sampled in 2002/2003, 1 in 2011 and 4 in 2017,

(ii) smaller slaughterhouses, performing a pre-treatment of their effluents before they were sent to a municipal WWTP: 6 slaughterhouses sampled in 2002/2003.

Antimicrobial resistance was investigated by disc diffusion method. Resistance determinants and integrase genes were amplified by PCR. During the 2017 sampling campaign, resistance to colistin and Critically Important Antibiotics (CIA) was specifically screened on antibiotic-supplemented plates.

Results. The percentage of resistant *E. coli* isolates among the total *E. coli* strains isolated was not different between wastewater, pre-treated and treated effluents. The season (autumn, winter, summer), as well as the year of sampling (before *versus* after Ecoantibio plans), also had no impact on this percentage. A variation was only observed according to the type of slaughterhouse. It was between 63% and 96% for the smallest slaughterhouses which were multispecies slaughterhouses. It was also high for plants where swine and veal calves were mainly slaughtered (from 80 to 96%). In contrast, it was lowest in plants where adult cattle was mainly slaughtered (from 7% to 46%). Resistance was mainly detected for tetracycline, ampicillin, streptomycin, sulfonamides and trimethoprim. During the 2017 sampling campaign conducted in cattle slaughterhouses, no strain resistant to colistin was isolated, and only a few CIA-resistant strains was detected: one *E. coli* isolate harbouring the $bla_{\text{CTX-M-15}}$ gene and *E. coli* isolates resistant to ciprofloxacin belonging to 5 distinct ERIC2 PCR (Enterobacterial Repetitive Intergenic Consensus) profiles.

Conclusion. The percentage of resistant *E. coli* isolates in slaughterhouse effluents was related to the category of domestic ungulates slaughtered in the plants.

P32

Plasmids carrying carbapenemase gene in Carbapenemase-producing *Enterobacteriaceae* isolated from companion animals in Korea

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Carbapenemase-producing *Enterobacteriaceae* (CPE) are resistant to the carbapenem class of antibiotics which are considered as the drugs of last resort for human infections. It is known that most of the treatment of companion animals with clinical sign depends on human antibiotics, and even carbapenems are used in severe cases. Since the first detection of carbapenem-resistant *Escherichia coli* from domestic companion animals in Korea, 2017, the seven CPEs among carbapenem-resistant *Enterobacteriaceae* (CRE) were collected from fecal samples of companion animals between 2019 and 2022. Whole genome sequencing analysis was performed using Nanopore and Illumina platforms in order to characterize them under genomic level. Plasmids harboring the carbapenemase genes were identified from the IncX3 plasmids in 5 strains and the IncFIA plasmids in 2 strains, respectively. A $bla_{\text{NDM-5}}$ gene was detected in 6 CPEs and an IncX3 plasmid (54,805 bp) carrying $bla_{\text{KPC-2}}$ was found in CPE isolated from asymptomatic companion dog in 2022. IncX3 plasmids (46,161 bp) carrying $bla_{\text{NDM-5}}$ in 4 CREs were identical to the plasmids reported in 2017. Because of concerns about close contact between humans and companion animals and the possibility of cross-species transmission, a multifaceted surveillance of antibiotic resistant microorganisms in companion animals is required.

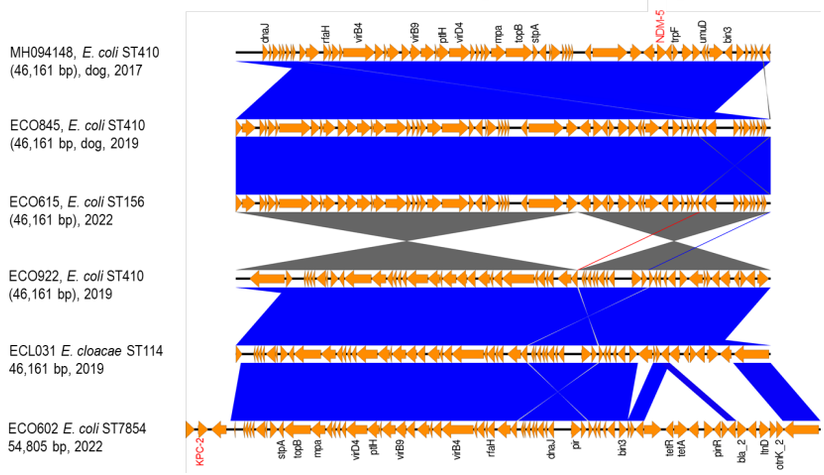

Fig. 1. Comparison of IncX3 plasmids carrying carbapenemase genes in companion animals

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P33

Comparison of geno- and phenotypic characteristics of *E. faecalis* and *E. faecium* with whole genome sequencing results

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Background, Enterococci are a group of bacteria of significant importance as they cause bacterial infections in humans. It is important to study this bacteria derived from dairy products, and enrich the presently insufficient data on the antimicrobial resistance and virulence factors.

Objectives, The aim of the study was genotypic characterization of archival strains obtained from milk and dairy products and comparison of the results with those obtained by phenotypic methods and classical PCR.

Methods and Results, A total of 36 strains from milk and dairy products were analyzed. The identification of *Enterococcus faecalis and Enterococcus faecium* species selected antibiotic resistance genes were detected using polymerase chain reaction. Panel of 18 antibiotics was performed using the broth dilution method. Deoxyribonucleic acid (DNA) isolation for sequencing was performed using the Maxwell Rapid Sample Concentrator kit. Quantitative and qualitative DNA assessments were performed and libraries created using the KAPA Hyper Plus kit. Sequencing was performed on the Nextseq instrument using a 2 × 150-bp paired-end protocol. Phenotypic resistance to tetracycline, kanamycin, streptomycin, erythromycin, chloramphenicol, tylosin and single strains resistant to vancomycin, gentamicin, teicoplanin and ciprofloxacin was determined. The tested virulence genes in most cases overlapped with the results of phenotypic resistance, with the exception of isolates sensitive to vancomycin (with *VanA* gene), erythromycin, tetracycline. Resistant straines lacked the *vgaA* and *vatD* gene. *E. faecalis* and *E. faecium* tetracycline-, erythromycin- and chloramphenicol-resistant genes found in the ResFinder 4.1 database were associated with resistance phenotypes.

P34

Raw milk and cheeses made from raw milk as a potential source of microbiological hazards

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Background, Raw milk and cheeses can be transmitters of many microorganisms that are dangerous to humans. Food borne pathogens can come from animal and the food production environment.

Objectives,
The aim of the study is to assess the occurrence and hazard characterization associated with the presence of *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp. and verotoxic *Escherichia coli* in raw milk (cow, sheep, goat milk). In addition, the research included the occurrence of enterotoxic positive staphylococci (CPS) and methicillin-resistant staphylococci (MRSA) in raw milk and cheese.

Methods and Results,
The study was carried out on the basis of the screening immunoenzymatic method with the use of mini Vidas analyser, and positive samples were confirmed with the reference methods. Pheno- and genotypic characterization of isolates were performed. Culture, biochemical, minimal inhibitory concentration and polimerase chain reaction methods were used.
In 2019-2022, a total of 1,075 bulk milk samples and cheeses from dairy farms from all over Poland were analyzed. The presence of *Listeria monocytogenes*, *Campylobacter* spp,, *Salmonella* spp. and verotoxic *Escherichia coli* was found. The percentage of the positive samples was 8% for *Listeria monocytogenes*, 3% for *Campylobacter spp.,* 0,1% for *Salmonella* spp. and 0,9% for *E. coli* O157. More than half of the samples were contaminated with CPS. Among the CPS, the presence of enterotoxigenic strains and MRSA was confirmed. Some strains of CPS showed the presence of staphylococcal enterotoxin genes encoding classic staphylococcal enterotoxins SEA, SEB, SEC and SED. The *seg*, *ser*, *sei*, *sej* and sep genes were also detected.

P35

ESBL gene dynamics of *Enterobacteriaceae* populations in the human-animal-environment interface

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Antimicrobial resistance (AMR) is a growing concern for both human and animal public health. The interaction between these two compartments and the environment has long been considered crucial in epidemiological and ecological studies. Extended-spectrum β-lactamases (ESBLs) are clinically relevant AMR mechanisms, given their capacity to degrade cephalosporins, a β-lactam class considered essential for the treatment of complicated infections caused by multi-drug resistant (MDR) pathogens, including *Enterobacteriaceae* species. Hence, this work aims to investigate the ESBL gene dynamics from a One Health perspective within a small municipality in the South of Spain. A total of 250 samples were taken from: pig farms (10 farms, 110 samples), hospital patients (35 samples) and water sources, both waste 2 WWTPs, 18 of WWTP samples; 11 community wastewater canalizations, 33 wastewater samples) and natural (3 rivers, 36 river samples, 3 underground wells, 18 samples). Total *Enterobacteriaceae* isolates were counted on MacConkey agar plates. Likewise, ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates were counted and selected in ESBL Brilliance plates, and identification was confirmed by MALDI-TOF. Minimal Inhibitory Concentration (MIC) to multiple antibiotic classes were assessed by broth microdilution with EUVSEC3 Sensititre plates. Whole Genome Sequencing (WGS) data from Illumina and Nanopore technologies were analysed by Unicycler, ABRicate and Roary, among others, in order to study genome structure, resistome/plasmidome content and pangenome structure, respectively. Total *Enterobacteriaceae* counts in pig faeces remained stable among farms, while the amount of ESBL-producing *E. coli* varied greatly. The same applied for extensive and intensive farms, with the latter presenting significantly higher levels of resistant bacteria (Mann-Whitney-Wilcoxon test, p-value = 5.329e-06). In water samples, a direct correlation was found between the presence of *Enterobacteriaceae* isolates and the amount of ESBL-producing bacteria, suggesting an effect of geographical distribution and faecal contamination on AMR levels. When selecting ESBL-producing isolates (evidenced by the high MICs against ampicillin, cefotaxime and ceftazidime,) resistance to chloramphenicol, trimethoprim and sulfamethoxazole were found as well. Preliminary WGS analyses revealed that ESBL-producing *E. coli* isolated from human patients belonged predominantly to ST131, a highly virulent, highly successful clone. These isolates carried mainly *bla*CTX-M-15 and *bla*CTX-M-27, highly prevalent and relevant ESBL genetic variants. Such AMR genes were associated to IncFII and IncFIB plasmids, respectively, pointing out their transmission potential through plasmid dissemination. Moreover, a CTX-M-15-producing ST131 isolate was found in the municipal WWTP, which suggests the community spread of this clinically relevant clone. Subsequent genomic analyses will uncover the resistome-plasmidome associations and the pangenome structure of the whole ESBL-producing *E. coli* and *K. pneumoniae* populations, as well as their relationship with the phenotypic profiles and epidemiological context. These will lead to an indepth understanding of the ecological forces governing intra- and inter-compartment AMR dynamics and the establishment of regulatory measures to preserve regional public health.

P36

NDM-producing Enterobacterales in Environment of Broiler Farm Rapidly Colonized in chicks

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Carbapenems play an important role in clinical medicine by serving as "last resort" antibiotics in treatment of serious infections caused by multidrug-resistant Gram-negative bacteria. Therefore, the emergence and global spread of New Delhi metallo-β-lactamases-producing carbapenem-resistant *Enterobacterales* (NDM-CRE) has become one of the most urgent global health threats. Usage of carbapenems in food-producing animals is prohibited. However, detection of NDM-CRE in farm animals is increasingly common. Our previous study revealed the long-term persistence of NDM-CRE in three floor-rearing chicken farms in Anhui province, China. To identify the risk factors associated with the persistence of NDM-CRE in these farms, we investigated the epidemiological characteristics of NDM-CRE in one broiler farm by testing samples collected within two complete breeding periods of two batches of broilers during April to August 2021. We collected a total of 3244 samples during the vacancy and raising periods in the farm, including 1030 chicken faeces (CF), 1841 environmental samples collected inside the chicken house (EI) and 373 outside the chicken house (EO). After enriching in LB broth, the samples were spread onto MAC agar plates containing 0.5 mg/L meropenem. One to three colonies (one colony per morphological type) from each plate were selected and PCR was performed to screen for the presence of the bla_{NDM} genes. The results showed that, during the vacancy period, 15.92% environmental samples were positive for NDM-CRE, and that such strains were rapidly acquired by chicks, for which the detection rate of NDM-CRE in faecal samples was up to 60% within 6 hours of being transferred to the farm; the rate continued to rise in the following 72 hours, reaching a peak in around 2 weeks (100%). The rate of carriage of NDM-CRE in the chicken fell afterwards but remained stable and relatively high prior to being sold in markets (65%). Three NDM variants were identified, namely NDM-5(n=178), NDM-1(n=22), and NDM-13(n=16). The predominant NDM-CRE species among the samples were *Escherichia coli* (CR-EC, 145/216) and *Klebsiella pneumoniae* (CR-KP, 36/216). Both clonal and horizontal transmission of bla_{NDM} were observed in both CR-EC and CR-KP strains isolated from chicken and environmental samples during the vacancy and raising periods. Several common sequence types (STs), such as CR-EC ST9388 and CR-KP ST1758 which carried plasmids of the same bla_{NDM}-IncHI2 type and exhibited a high degree of sequence similarity, were identified. In summary, our findings show that chicks can be rapidly colonized by environmental NDM-CRE in the farm; such strains can rapidly spread and persist among the animals and in the farm environment during raising period. It is important to implement "all-in/all-out" management policy and carry out thorough cleaning and disinfection in broiler farms to prevent NDM-producing bacteria from being able to persist and disseminate in chicken farms.

P37

Genomic analysis of antimicrobial-resistant *Escherichia coli* in a gull colony in the Czech Republic

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Wildlife plays an important role in the spread of antibiotic resistance and is one of the main reservoirs for the emergence and dissemination of antibiotic-resistant bacteria in the environment. Migrating animals are of great importance as they may spread antibiotic resistance in different geographical areas. This study aimed to investigate the presence and dynamics of antibiotic-resistant *Escherichia coli* in Caspian gulls (*Larus cachinnans*) from the Czech Republic and their potential role in the spread of antibiotic resistance.

Cloacal samples from birds breeding in the Nove Mlyny water reservoir were collected in June 2018 (n=72) and May 2019 (n=45) and 6 water samples were taken from varios locations the reservoir in 2019. Isolates were selected on media with ciprofloxacin (0,05 mg/ml), cefotaxime (0,2 mg/ml) or colistin (3,5 mg/ml). Resistant *E. coli* isolates were whole genome sequenced using Illumina. In addition, 1393 isolates from EnteroBase were analysed phylogenetically since they belonged to the same sequence type (ST) as our gull isolates. The search on EnteroBase was restricted to the Czech Republic and neighbouring countries, where the gulls were observed to migrate according to our telemetry data. The phylogenetic tree, which comprised 1534 isolates, underwent assessment and recalculation, with only isolates that differed by up to 100 SNPs from ours being included.

A total of 176 *E. coli* isolates were obtained, out of which 141 were sequenced. In 2018, a high occurrence (79 %, 57/72) of ESBL/AmpC-producing *E. coli* was observed compared to 38 % (17/45) in 2019. The predominant clonal lineage in 2018 was ST11983 (37/57 isolates), which carried a F34:A-:B- plasmid with *bla*CMY-2 gene. In year 2019, ST2325 (12/17 isolates) carrying F2:A-:B- plasmid and *bla*TEM gene was the most common. This ST was also found in water samples obtained this year. High proportions of several STs commonly recognized as internationally high-risk lineages, such as ST10, ST58, ST88, ST117, ST648, or ST74415, were found in the samples from both years as well as in the water sample. According to the results of the phylogenetic analysis, certain STs were detected in multiple sources, including humans, and a subset of these STs were found to be closely related to isolates recovered from gulls.

Our findings showed that the presence of *E. coli* strains resistant to antibiotics can fluctuate significantly over time in synanthropic birds, highlighting the importance of longitudinal studies. We identified both a temporary dominant lineage and persistent sequence types. The similarity between gull isolates and those in EnteroBase underscores the importance of assessing the potential risks associated with migratory birds as well as with anthropogenic influence on nature.

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P38

Impact of biosecurity and hygiene measures on different routes of introduction and persistence of antimicrobial resistance in pig and broiler farms.

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Evidence suggests that antimicrobial resistant (AMR) bacteria and genes can persist and spread within and between livestock premises even in settings where antibiotic usage is low or absent. To allow for effective surveillance and control strategies of resistant organisms in the livestock sector, it is key to identify main entry points and test current routine cleaning protocols. This study aimed to assess the presence of AMR *E. coli,* as an indicator organism, in livestock housing and in main farm inputs. To evaluate the efficacy of cleaning and disinfection protocols applied in different sectors, environmental swabs were collected from pig and broiler farms. On each site, one building that was used to house livestock was sampled three times – while populated by one group of animals, after being vacated and cleaned, and finally after introduction of a new production group. Swabs were collected from indoor surfaces in different sections of the houses, as well as from outside areas surrounding the entrance to the buildings. Water, bedding, feed, and replacement animals were sampled to evaluate their potential role in introducing contamination. To date, 299 confirmed *E. coli* isolates have been isolated from 50 broiler house samples and 303 isolates from 54 finisher pig pens samples. These were tested for susceptibility to 10 antibiotics through breakpoint sensitivity testing: ampicillin, cefotaxime, ceftazidime, ciprofloxacin, tetracycline, sulfamethoxazole, trimethoprim, streptomycin, apramycin, florfenicol. Overall, 61% of isolates from the broiler house and 24% of the finisher pig pens were resistant to three or more antibiotic classes and identified as multidrug resistant (MDR). Most of the *E. coli* isolates from the broiler farm were resistant to ampicillin (77%), sulfamethoxazole (71%) and tetracycline (57%). A similar resistance profile - but at lower levels - was found in the pig housing isolates: ampicillin (26%), tetracycline (15%) and sulfamethoxazole (11%). The percentage of *E. coli* isolates that were MDR at each of the three visits was 69%, 45% and 70% respectively for the broiler house, and 32%, 16% and 23% respectively for the pig house. After cleaning, all indoor samples from the broiler house - except for one floor swab - tested negative for *E. coli,* while isolation was possible from all samples of the pig housing throughout the sampling period. No statistically significant difference was observed in the proportion of MDR isolates between the three broiler farm visits. However, there was a significant reduction in the percentage of MDR *E. coli* after depopulation and cleaning of the pig pens ($P = 0.009$). These results suggest that the cleaning and disinfection strategy applied in the broiler sector, may help to reduce carryover of *E. coli* contamination between production groups in comparison to the pig sector. However, MDR *E. coli* appear to play a role in residual AMR contamination in livestock housing also in the context of relatively successful cleaning and disinfection. Genetic analysis on the collected MDR isolates will provide more information on their potential persistence and eventual genetic features preventing their elimination. Pilot data identified replacement animals as a key source of MDR *E. coli,* suggesting the need for surveillance in hatcheries and monitoring of pigs - whether reared on farm or externally sourced - before introducing them in finishing units.

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Interventions to control the dynamics of antimicrobial resistance from chickens through the environment: ENVIRE

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The overall objective of the project ENVIRE is to contribute to the reduction of the selection and the spread of antimicrobial resistance in broiler chickens and from chicken farms to the environment, and ultimately to humans. Different intervention studies will investigate the potential of various on-farm measures: i) Antibiotic-free chicken raising, ii) Phytotherapy as alternative for antibiotics, iii) *E. coli* vaccination, iv) Application of bacteriophages, v) Treatment or storage of manure, vi) Depollution of farm effluents to remove antibiotics and their residues. Experimental studies will be complemented by field studies whenever possible. Focus will be laid on ESBL *Escherichia coli* and *Enterobacteriaceae* and on resistance against fluoroquinolones as well as colistin. A quantitative risk assessment model will be developed and used to assess the effectiveness as well as potential synergistic effects of the interventions, to reduce human exposure via the foodborne, occupational and environmental pathways. Data already available for the participating countries will be included in the model, and new, essential data will be generated within the studies. As a result, specific as well as general interventions will be identified that have the potential to reduce AMR in chicken and in the environment of chicken farms for Europe and Tunisia. To achieve this, six working groups from Germany, France, Lithuania, Poland, and Tunisia, bundle their leading expertise for the respective issue.

P40

Persistence of multidrug resistant *Escherichia coli* in chicken breeding environment

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Antimicrobial resistance (AMR) has decreased in French broiler production for the last 10 years, linked to lower antibiotics use. Nonetheless, animal gut and the breeding environment are still considered as reservoirs of resistant zoonotic bacteria. The aim of this study was to characterize the dynamic of AMR transmission and persistence in an experimental facility simulating a broiler production system with no antibiotic exposition.

Chickens from five families were followed over three generations and two breeding environments (meat production and lineage reproduction), and commensal AMR *E. coli* strains were isolated from feces at different weeks of age, as well as from building surfaces before flock entry. Among the ca. 600 non-redundant isolates collected, a subset of 128 multidrug resistant isolates (at least 4 antibiotic classes) were subjected to sequencing with lllumina and/or Oxford Nanopore technologies.

The vast majority of isolates (91%) belonged to six unrelated sequence types from phylogroups A (ST206, ST2701, ST93) and B1 (ST162, ST1611 and ST453), none being reported as major causes of human or animal infection. These ST were shared between chicken families, generations and sample type (feces or surfaces), but segregated by breeding environment: isolates of phylogroup A were associated with reproductive animals while isolates of phylogroup B1 were associated with production animals. The AMR gene repertoire of sequenced isolates showed a weak diversity, with several genes (*i.e. tet*(A), $bla_{\text{TEM-1}}$, *sul2*, *dfrA1*, *aadA1*) being shared by most ST. However, they were carried by independent plasmids, *i.e.* pIncF and pIncHI1 in ST2701 isolates, pIncZ in ST93 isolates, or pIncI1 in ST453 isolates.

Our results indicate that the transmission of AMR in animals of successive generations is mainly driven by the persistence of specific clones in our environment facility, rather than direct vertical transmission (from mother to offspring) or horizontal transmission of resistance plasmids.

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P41

Antimicrobial resistance in commensal *Escherichia coli* on Irish pig farms: a longitudinal study

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New European Union regulations restricting antimicrobial use (AMU) in livestock aim to minimise potential human exposure to antimicrobial resistant bacteria of animal origin. Nevertheless, a better understanding is required of how different levels of AMU during the production cycle affect the development of antimicrobial resistance (AMR) in indicator bacteria such as *Escherichia coli*. This is especially relevant to pig farming since it is typically the livestock sector with the highest AMU. This study sought to evaluate how AMR in *E. coli* evolves during the lifecycle of pigs on Irish farrow-to-finish farms with different levels of AMU.

Twelve Irish farrow-to-finish farms were selected according to their level of AMU in medicated feed (low, moderate, high). Ten litters of piglets were selected from each farm; pooled faecal samples were collected during each production stage (piglet, weaner, grower and finisher). Twenty *E. coli* isolates from each pooled sample were tested against a panel of 16 antimicrobials using EUCAST defined epidemiologic cut off values (ECOFF). For each of the six most prevalent resistance phenotypes, as well as for the multidrug resistant (resistance to ≥ 3 antimicrobial classes) and pan suseptible phenotypes, mixed effects logistic regression models were constructed using AMU practice and stage of production as explanatory variables.

The highest frequencies of resistance were to doxycycline, ampicillin, trimethoprim/sulfamethoxazole, chloramphenicol, gentamicin and the fluoroquinolones. Resistance to these agents was highest in weaners except for fluoroquinolones where it was highest in piglets. Similarily, multidrug resistance was highest, and pan suseptibilty was lowest, in isolates from the weaner stage. Resistance was lower on farms not using medicated feed (low AMU) compared to moderate or high AMU farms with the exception of fluoroquinolone and trimethoprim/sulfamethoxazole resistance. Resistance to amikacin or imipenem was not observed and resistance to the cephalosporins was low.

Stage of production and in-feed AMU practices influence the occurrence of AMR in *E. coli* in pig production. These findings have implications for strategies to control AMR at farm level.

P42

Investigation into the occurrence of AMR in *Escherichia coli* in wild birds

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Antimicrobial resistance (AMR) is a critical and growing problem worldwide. *Escherichia coli* is a commensal bacterium commonly present in the intestine of animals and people, and a useful indicator to monitor AMR circulating between hosts and the environment. There is evidence to suggest that wild birds can carry AMR-harbouring bacteria which they can spread through migration. Livestock feed, surface waters and pastures are all susceptible to contamination from wildlife excreta.

AMR harbouring *E. coli* have been isolated from many bird species, occupying diverse ecological niches, including ducks and geese, cormorants, birds of prey, gulls, doves, and passerines [1]. Gulls especially scavenge at wastewater treatment plants and landfill sites, and therefore can act as sentinels, mirroring AMR in people and possible transmission into the environment. Wild birds are also potential spreaders of AMR through their ability to migrate long distances in short periods of time, presence on pastures with livestock or access to livestock accommodation, and potential contamination of surface waters / environment.

In this study we have investigated the prevalence of AMR *E. coli* in over 100 wild birds across UK using selective media for the detection of ESBL/AmpC- and carbapenemase-producing *E. coli* [2]. We have also investigated the prevalence of *E. coli* resistant to two antimicrobials used in animal production in the UK and Europe, tetracycline and apramycin. Phenotypic and genotypic characterization to determine AMR profiles of *E. coli* that are circulating in the wild bird population across UK has been undertaken, with comparisons made to isolates recovered from other sources.

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P43

Influence of management factors on Austrian dairy farms and the prevalence of antibiotic resistance among ESBL/AmpC-producing *E. coli*

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A total of 51 dairy farms in four federal states in Austria were included in the project. Farmers were recruited by their local veterinarian. Antibiotic use data were provided from the veterinary practice software. Farmers were requested to complete an online survey of management factors. Faecal samples were collected directly from pre-weaned calves (< 6 weeks of age), weaned calves (> 6 weeks of age) and the freestall/tie-stall alleys of dairy cows. Faecal samples were collected from calves using rectal swabs, which were then pooled in the laboratory. Cow samples were collected using boot swabs.

For the detection of ESBL/AmpC-producing *E. coli*, incubated sample material was spread on a selective MacConkey agar containing 1 mg/l cefotaxime and incubated. A subculture was spread on selective culture medium (MacConkey agar containing 1 mg/l CTX) and incubated again. The pure culture was confirmed by MALDI-TOF. The presence of resistance in all pure cultures was checked using the Sensititre™ EU Surveillance (EUVSEC3) plate. Isolates that showed resistance to cefotaxime, ceftazidime or meropenem were re-screened for resistance using the Sensititre™ EU Surveillance ESBL (EUVSEC2) plate.

Overall, 50 farmers completed the online survey. Cephalosporins and penicillins were the most frequently used antibiotics on these farms. Waste milk was routinely fed to calves on 88% of farms. Blanket dry cow therapy was used on 26.9% of farms, while over 40% of farmers stated that they used antibiotic dry cow therapy only on symptomatic cows. From 190 faecal samples, 56 isolates (29.5%) were confirmed as ESBL/AmpC-producing *E. coli*. ESBL/AmpC-producing *E. coli* were found on 27/51 (52.9%) of dairy farms tested. The lowest prevalence of ESBL/AmpC-producing *E. coli* were found among samples from weaned calves (22.5%), while dairy cows (31.9%) and pre-weaned calves (31.9%) were equally high.

Statistical analyses demonstrated links between the presence of ESBL/AmpC-producing *E. coli* and the feeding of waste milk to calves in these herds. Farms where calf pens/hutches were washed and disinfected between restocking with new calves were more likely to have ESBL/AmpC-producing *E. coli* present than farms where pens/hutches were simply mucked out and straw replaced. This is likely to be indicative of a higher level of infection and general health issues leading to a need for disinfection, rather than a causal relationship of disinfection causing resistance.

P44

Human carriage of ESBL/pAmpC-producing *Escherichia coli* and *Klebsiella pneumoniae* in relation to the consumption of raw or undercooked vegetables, fruits and fresh herbs

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Background and objectives: Vegetables, fruits and herbs can be contaminated with ESBL-producing Enterobacterales. Although fresh produce appears to be less frequently colonized with antimicrobial resistant bacteria compared to meat products, their role as a reservoir might be important since many of these products are consumed without preparation, such as washing or heating. In this study, we investigated to what extend the consumption of raw vegetables, fruits and fresh herbs influences carriage rates of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* (ESBL-E/K) in the general population. Secondary, we assessed long-term carriage as well as changes in ESBL-E/K prevalence during the COVID-19 pandemic, by comparing the results to earlier findings in the same population.

Materials and methods: Between July and December 2021 participants sent in two faecal samples, three months apart, accompanied by a digital questionnaire. During the study period food frequency questionnaires were sent on a monthly basis. Faeces was cultured using selective enrichment and subsequent culture on selective agar. Phenotypically positive ESBL-E/K isolates were sequenced using Illumina short read sequencing. Long read sequencing (Oxford Nanopore) was performed in participants that carried the same ESBL gene and bacterial ST in both samples. Multivariate logistic regression models were established to assess the association between the average weekly consumption of specified vegetables, fruits and ESBL-E/K carriage.

Results: The ESBL-E/K prevalence based on the first faecal sample was 7.6% (41/537; 95% CI 5.7-10.2) and 7.0% based on the second sample (34/489; 95% CI 5.0-9.6). The multivariate models did not result in statistical significance for any of the selected fruit and vegetable types. Trends for increased carriage rates were observed for the consumption of bell pepper, celery and several types of berries (raspberry, blueberry, blackberry). *bla*$_{\text{CTX-M-15}}$ was the predominant ESBL/pAmpC gene among the participants, followed by *bla*$_{\text{DHA-1}}$. The most frequently found sequence types were *E. coli* ST 69, 10 and 131. The ESBL-E/K prevalence was comparable to a previous cross-sectional study of the same cohort performed in 2015-2017 (7.5%; 95% CI 5.6-10.1%). A comparison of the core genome showed that in six persons the ESBL-E/K was genetically highly similar to the bacteria found approximately five years earlier.

Conclusion: This population study showed that the contribution of the consumption of raw fruits and vegetables to ESBL-E/K carriage in humans in the Netherlands is probably low. In addition, we found indications for long-term carriage over a period of 5 years and demonstrated that the effect of the COVID-19 pandemic and its containment measures on ESBL-E/K carriage rates in the general population is small.

P45

Mobile tigecycline resistance gene found in live bird markets in Dhaka, Bangladesh

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Multidrug-resistant pathogens present a significant risk to public health. Indicator species such as *Escherichia coli* can be used to assess the risk to consumers of the spread of AMR around a population. There is currently a limited understanding of AMR characteristics in Bangladesh, as well as in live bird markets. Caecal contents were collected from Sonali and Broiler chickens from a live bird market in Dhaka, Bangladesh. Samples were collected in 2018 (108) and 2020 (109) and isolated on MAC. A selection of 83 isolates were sent to the UK for broth microdilution assays to determine the MIC using commercially available broth microdilution plates. WGS was performed on all isolates to identify AMR genes as well as evaluate phylogenetic similarity based on core genome SNPs. 77/83 isolates were MDR, with the most common pattern of resistance to tetracycline, trimethoprim, sulfamethoxazole, ampicillin, and ciprofloxacin. All ciprofloxacin resistant isolates contained *qnrS* genes or *gyrA* and *parC* chromosomal mutations. 39/83 of isolates were resistant to azithromycin associated with *mphA* genes. Four isolates were ESBLs, two with $bla_{CTX\text{-}M\text{-}55}$, two with $bla_{CTX\text{-}M\text{-}65}$. Two isolates were AmpC phenotypes with $bla_{DHA\text{-}1}$ genes. Seven isolates were colistin resistant, confirmed with the presence of *mcr1.1* genes. Two isolates were resistant to tigecycline, one with an acquired *tetX4* gene. The presence of MDR E. coli in live bird markets presents concern to public health. The discovery of *tetX4* has not yet been characterised in Bangladesh and is therefore significant. Finding that 8% of isolates were colistin resistant with *mcr1* genes is also noteworthy given colistin is prohibited in animal use. An increase in food safety awareness and improved AMU stewardship in farms and in hospitals may contribute to a risk reduction. Continued surveillance at slaughter can allow monitoring of trends and impact of efforts to reduce AMR.

P46

One Health approach applied to the following up of antimicrobial resistance dissemination in freshwater fishfarms at the watershed level

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A research project (Resist3A) aimed to investigate the AMR dissemination upstream and downstream of two trout fish farms, located respectively at the source (FF1) and at the mouth (FF2) of a river impacted by agricultural, terrestrial farming and wastewater treatment plant.
During 17 months, every two weeks, water samples were collected upstream and downstream two fish farms and biofilm samples were collected in a same fishpond per farm. Abiotic environmental parameters (pH, conductivity…) were measured too. Enumeration of *E. coli*, *Aeromona*s spp. and *Pseudomonas aeruginosa* was performed using culture methods on the 144 water samples and 72 biofilm samples collected.
Antimicrobial susceptibility testing of a selection of *E. coli* (n=142), *Aeromonas* (n=355), and *Pseudomonas aeruginosa* (n=123) isolates was performed using agar diffusion method.
The proportion of *E. coli* isolates, which were susceptible to all tested antimicrobials, was higher in water than in biofilm (92.7% vs 89%). No isolate was resistant to carbapenems or extended-spectrum beta-lactams. No multidrug-resistant isolate was detected. Only 13 isolates were resistant to one or two classes of antibiotics.
The *Aeromonas* isolates collected were collected from water samples (n=172) and from biofilm (n=183). From the source to the mouth in water sample, no decrease of susceptibility was observed for oxolinic acid, florfenicol, oxytetracycline, ceftazidime and meropenem. At the opposite, a slight reduction of susceptibility was observed for enrofloxacin. Isolates collected in the biofilm of the FF1, showed a reduced susceptibility to oxytetracycline and oxolinic acid. No reduction of susceptibility was observed among isolates collected in the biofilm of the FF2.
On the contrary, of the results observed for *E. coli*, the proportion of *Pseudomonas aeruginosa* susceptible to all the 13 antibiotics tested was higher in biofilm than in water (100% vs 74.5%).
These results confirm the importance of an approach including several compartments of the environment (water, biofilm), and three bacteria models allowed us to have a broader view of the AMR dissemination.

P47

Assessment of the diffusion of AMR in aquatic environments: specificity of river fish farming

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Antimicrobial resistance (AMR) is now recognized as among the top 10 threats to global health, with current trends in resistant infections in humans and livestock pointing toward a potential postantibiotic era (Liguori, et al., 2022). The aquatic environment is considered as a hot spot for the dissemination of AMR, due to its direct link with the aquatic environment, the issue of monitoring AMR in aquaculture arises.

According to Liguori and collaborators (2022), a fundamental stumbling block to the advancement of AMR monitoring of water environments is a lack of agreed upon targets and standardized methods, including a lack of benchmarking and threshold data to inform evolutionary, epidemiological, and other risk modeling efforts.

AMR monitoring in aquaculture is concerned by the same problematics. Using trout fish farming as a case study, we will provide some answers to the following questions:

Where? Fish farming depends on the quality of the water received. Sampling strategy should then include data collected upstream and downstream of the fish farming.

What? What kind of samples water or/and biofilm or/and sediment? A sample should be easy collected and always available.

Who? *E. coli* is a well-characterized bacterium used for the monitoring of AMR in humans and livestock (in national surveys, at the European level and elsewhere), as well as for the monitoring of water quality (as a fecal contamination indicator). Could *E. coli* be used? What alternatives can be offered?

How? Currently AMR monitoring plans is based on culture depend methods and phenotypic criteria. Nevertheless, the use of genetic indicator or genomic approach are under progress.

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P48

Orf121 carried by the IS*91* insertion sequence negatively influences its mobility

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Prokaryotic insertion sequences (IS) contribute to bacterial multidrug resistance. IS*91* is usually associated with virulence and antibiotic-resistance genes, but its contribution to their dissemination is unclear. IS*91* belongs to an atypical IS family with a HUH transposase mediating transposition events using a rolling circle transposition mechanism1. This IS family displays two functionally distinct ends: transposition initiates at the so-called *ori*IS end and terminates at the *ter*IS end. Nevertheless, *ter*IS could not be recognized, leading to the mobilization of an adjacent DNA fragment ("one-ended transposition")2. Unlike other members of the family, IS*91* carries upstream the *tnpA* transposase gene, a small ORF potentially encoding a 121 amino acids polypeptide: Orf1212. The termination codon of *orf121* overlaps the initiation codon of *tnpA*, suggesting that the expression of both genes may be translationally coupled via Termination-Reinitiation mechanism.
We investigated the role of Orf121 in the *in vivo* transposition of IS*91* using a genetic system based on the mating-out procedure in *Escherichia coli*. Furthermore, we have detected two potentially functional promoters (P*orf121* and P*tnpA*) that could drive the expression of the two ORFs.
We showed that co-expressing Orf121 (*in cis* or *in trans*), in addition to the transposase gene, strongly decreased both the IS*91* transposition frequency and the one-ended transposition rate; these results indicate the Orf121 polypeptide may exert a negative effect on the *in vivo* transposition of IS*91* and that it may be required for accurate recognition and cleaving of the *ter*IS end. Then, we mapped more than 1000 *in vivo* IS*91* insertion sequences indicating that the target site specificity of IS*91* does not depend on the presence or absence of Orf121. Furthermore, Orf121 does not tetranucleotide the *ter*IS cleavage site. Lastly, we showed that the activity of the P*orf121* promoter is significantly higher than that of the P*tnpA* promoter.

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P49

Resistance of *Salmonella* to heavy metals, a key element for dominance in the agro-food industry in Europe?

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For the last 50 years, the success of the epidemic clones of the *Salmonella* Typhimurium and its monophasic variant (*S*. 4,[5],12:i:-) (DT9, DT204, DT104 and DT193) was mostly attributed to their antimicrobial resistance profile. The recent discovery of a genetic island SGI-4 carrying resistance genes to copper, arsenic and silver and its role in the emergence of the European clone (DT193 or ST34) suggested that other factors, such as heavy metal resistance, can favorise the selection of epidemic clones enabling them to survive along the agro-food production chain until humans [1, 2].
In this study, the heavy metal resistance genes from the BacMet database were screened in 168 genomes belonging to the 11 *Salmonella* serovars prevalent in agro-food sectors in France. The minimal inhibitory concentration (MIC) and growth kinetics were performed in concentrations of copper relevant to farm environments for four strains carrying (or not) operons *pco* and *sil*, identified as coding for copper resistance in the SGI-4. The intracellular copper concentration of these strains was quantified by mass spectrometry and the expression of 18 genes coding for copper resistance was explored by reverse transcription PCR.
Within the 52 genes identified, the ones involved in copper metabolism and resitance were prevalent within the 168 genomes analyzed. Interestingly, *S.* Senftenberg carry 25 of the 89 SGI-4 genes with both *pco* and *sil* operons and some *S.* Agona strains (n=3/10) encoded *sil* cluster, confirming the mobile nature of these elements. The presence of *pco* and *sil* clusters did not affect the MIC, growth kinetics and mass spectrometry analyses in aerobiosis. However, in anaerobiosis, strains carrying *pco* and/or *sil* clusters were advantaged with both higher growth resistance rate and greater biomass production suggesting that copper resistance likely plays a role during intracellular life of *Salmonella* in the host. The use of copper as feed complement could modify the intestinal concentration of this compound in the hosts and exert selective pressure on microorganisms able of resisting its toxicity. The transcriptomic analysis confirmed the involvement of genes described and predicted as involved in copper resistance, especially *sil* operon. Finally, our results underlined that the copper resistance appears to be a key factor in the survival of *Salmonella* in the host and suggest that relationship between heavy metal resistance and antibiotics for *Salmonella* would deserves further study.

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P50

Effects of subinhibitory vancomycin and gentamycin concentrations on biofilm formation by *Staphylococcus aureus*

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Objectives: To examine the effect of sub-inhibitory concentrations (sub-MIC) of antibiotics on biofilm formation by *Staphylococcus aureus* (*S. aureus*) clinical isolates.
Methodology: Twenty *S. aureus* isolates were investigated using conventional microbiological methods. The susceptibility of uropathogenic staphylococci to vancomycin was performed by broth microdilution following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). The biofilm-forming ability of *S. aureus* isolates treated and untreated with vancomycin and gentamycin sub-MICs was assessed in vitro using the tissue culture plate (TCP) method combined with a crystal violet staining assay.
Results: According to the tissue culture plate approach, all strains of *S. aureus* have shown the capacity of biofilm formation, 29% of the isolates were strong biofilm producers, 57% of the isolates were moderate biofilm producers, and 10% of the isolates were weak biofilm producers.
Different changes in the ability to form biofilms were detected in *S. aureus* isolates treated with vancomycin and gentamicin sub-MIC concentrations. MIC/4 concentration of vancomycin had an inhibitory effect on biofilm formation in 92.96% of isolates, while biofilm formation increased in 31.3% of isolates at the MIC-16 concentration. MIC/4 and MIC/8 concentrations of gentamycin had inducing effects on the rate of biofilm formation in 54.55% and 83.33% of isolates, respectively.
Conclusion: The findings of this research demonstrated that the reduction and induction effects on biofilm formation ability are antibiotic-dependent and even vary from strain to strain.

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P51

KSA-1, a new Ambler class A extended-spectrum β-lactamase from a plant bacterial enterobacterial species

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Several bacterial species belonging to the *Gammaproteobacteria* possess intrinsic class A β-lactamase genes in their chromosome that may represent sources of further dissemination and acquisition in other Gram-negative species.
Here we characterized the KSA-1 class A β-lactamase which gene was identified from the chromosome of an environmental *Enterobacterales*, namely *Kosakonia sacchari* (formerly *Enterobacter sacchari*), recently identified as a progenitor of an MCR-like colistin resistance determinant.
Methods
In-silico analysis using the GenBank database identified a class A β-lactamase gene in the chromosome of *Kosakonia sacchari* SP1 (GenBank accession no. WP_065368351). The corresponding protein KSA-1 shared 63% amino-acid identity with intrinsic CKO-1 from *Citrobacter koseri* and 53% with TEM-1. The bla_{KSA-1} was amplified, cloned into plasmid pUCp24 and expressed in *E. coli* TOP10. MICs and kinetic parameters were obtained from the purified enzyme.
Results
K. sacchari SP1 was isolated from a stem of sugar cane cultivar grown in China. Once produced in *E. coli*, KSA-1 showed a typical ESBL resistance pattern with high level resistance to penicillins and temocillin, with a significative reduction of the MICs value in presence of class A ß-lactamase inhibitors (clavulanic acid, tazobactam and avibactam). The strain was resistant to cefoxitin, ceftazidime, aztreonam, but remained susceptible to cefotaxime.
Kinetic assays were performed using a purified extract of KSA-1, showed a high hydrolysis rate for benzylpenicillin and piperacillin. Determination of inhibitory constants showed IC_{50} values of 2.2, 3 and 1.8 nM for clavulanic acid, tazobactam and avibactam, respectively. Analysis of sequences surrounding the bla_{KSA-1} gene did not reveal any mobile element that could have been involved in the acquisition of this β-lactamase gene.
Conclusion
KSA-1 was characterized as a class A extended spectrum β-lactamase (ESBL), distantly related to known extended-spectrum ß-lactamases, with a high activity against temocillin. The bla_{KSA-1} gene was considered as intrinsic to the species, that latter being therefore a putative progenitor of an ESBL encoding gene.

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Hidden antimicrobial resistances in *Vibrio parahaemolyticus*: Environmental bacteria as sources or vehicles for the spread of plasmid encoded clinically important antimicrobial resistances

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Antimicrobial resistance (AMR) is on a rise and challenges global One Health increasingly. The emergence of different antimicrobial-/biocide resistances in the individual One Health compartments (environment, animals and humans) is usually associated with an adaption of the bacteria against prevailing selection pressures (antimicrobials, residues or biocides). Mobile genetic elements (MGEs) carrying transmissible resistance determinants are common and are widely spread among bacteria. Because of their localization on plasmids, bacteriophages and insertion sequences, they can also be transmitted between bacteria by different mechanisms during horizontal gene transfer. Investigation and monitoring on the emergence of transmissible resistances is important, but sometimes their phenotypic development is masked by their hosts. Recently, a *V. parahaemolyticus* isolate was notified to carry a carbapenemase-producing plasmid, which only lead to slightly increases MIC values for carbapenems in *Vibrio* spp. Nevertheless, the location of the resistances on a self-transmissible plasmids results to a high resistance phenotype against different carbapenems after natural transmission into a broad range of *Enterobacteriaceae* isolates. The properties and the genome of the plasmid associated with this hidden resistance phenotype as well as the genotypic and phenotypic features of the *V. parahaemolyticus* isolate will be presented and discussed. The data clearly showed that some bacteria can acquire and mask resistance plasmids, which were further spread to clinically relevant genera associated with severe nosocomial infection in human.

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Staphylococcus aureus plasmids of animal origin, a vector of antibiotic resistance

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Staphylococcus aureus is a human and animal opportunistic pathogen that has demonstrated the capacity to develop resistance to a wide range of antibiotics. Its ability to acquire antibiotic resistance genes (ARGs) is closely associated with the large proportion of mobile genetic elements (MGEs) present in its genome (between 15 to 20%) and its capacity to exchange genetic material with its environment1. We recently conducted an *in silico* analysis of MGEs and their linked ARGs on a dataset of 1257 *S. aureus* genomes isolated from animals, obtained from the NCBI database. As this analysis suggested the central role of plasmids in the dissemination of antibiotic resistance, our aim here was to provide further evidence from the field by characterizing 340 *S. aureus* isolates collected from different animal species by the Resapath network2 in France from 2010 to 2021. Their antibiotic resistance genes were detected by PCR, their plasmid content was identified by PFGE and finally the plasmid/resistance gene associations were established with Southern Blot.
Data showed that almost all isolates (304/340, 89.4%) carried at least one resistance gene, of which 60% contained *blaZ* gene (beta-lactam resistance), 55% *tet(M)* (tetracycline resistance) and 40% *aac(6')-aph(2'')* (aminoglycoside resistance). The diversity of ARGs in these *S. aureus* field isolates was similar to that found in our *in silico* analyses. A large proportion of isolates (55%) contained at least one plasmid, with an average of three plasmids per isolate. The plasmid sizes varied between 2kb and 160kb, but isolates mostly carried plasmids whose size was either 25kb-35kb (27%) or 4.3kb (21%). Southern Blot analyses showed that 40% of *S. aureus* plasmids carried at least one of the four ARGs *aadD*, *erm(C)*, *str*, or *tet(K)*. Contrarily to *in silico* data, *tet(M)* was only found localized on the chromosome. A large diversity of ARG/plasmid associations (42 different profiles) was observed. Each gene was found to be more commonly associated with a specific size of plasmid: *str, tet(K)* and *erm(C)* were located on small (<5kb) plasmids while *aadD* was found either associated with medium-size plasmids (25kb) or associated with *erm(C)* on a small 9kb plasmid.
This study confirmed that plasmids play an important role in antimicrobial resistance in *S. aureus*, as suggested by *in silico* analyses. Short- and long-read sequencing are ongoing on a subset of isolates carrying representative patterns of plasmids in order to refine their characterization. Further research is needed to uncover the mechanisms behind the wide dissemination of these plasmids and to explain their epidemic success.

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P54

Staphylococcus chromogenes from bovine sub-clinical mastitis in Sweden

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In Sweden bovine subclinical mastitis is commonly caused by *Staphylococcus chromogenes*. However, knowledge on genetic relatedness, virulence factors and genes encoding antibiotic resistance is overall scarce for *S. chromogenes*. The current study therefore performed genome sequencing and bioinformatic analysis on 105 *S. chromogenes* isolates. The isolates had previously been obtained from Swedish dairy cows with subclinical mastitis in a prior observational study [1]. Isolates were characterized using a 7 locus multi locus sequence typing (7-MLST), core genome analysis, detection of genes encoding AMR, and genetic detection of prospective virulence factors. The isolates belonged to 47 STs, with the most common being ST6 and ST109, and were defined to belong to 7 distinct core genome clusters. Isolates were generally susceptible to antibiotics with only the genes *blaZ* and *str* identified in a limited number of isolates. *S. chromogenes* were on average positive for around 30 prospective virulence factors, with a variation of types and numbers of factors identified in clusters and STs.

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P55

Characterization of an *optrA*-harbouring unconventional circularizable structure located on the novel integrative and conjugative element ICE*Ssu*HN38 in *Streptococcus suis*

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Objectives: To identify the novel genetic elements involved in the horizontal transfer of the oxazolidinone/phenicol resistance gene *optrA* [1] in *Streptococcus suis*.
Methods: Whole genome DNA of the *optrA*-positive isolate *S. suis* HN38 was subjected to WGS via both Illumina HiSeq and Oxford Nanopore platforms. MICs of several antimicrobial agents were determined by broth microdilution. PCR assays were performed to identify the circular forms of the novel integrative and conjugative element (ICE) ICE*Ssu*HN38, but also the unconventional circularizable structure (UCS) excised from this ICE. The transferability of ICE*Ssu*HN38 was evaluated by conjugation assays.
Results: *S. suis* isolate HN38 harbored the oxazolidinone/phenicol resistance gene *optrA*. The *optrA* gene was flanked by two copies of *erm*(B) genes in the same orientation, located on a novel ICE*Sa*2603 family-like ICE, designated ICE*Ssu*HN38. PCR assays revealed that a novel UCS [2] carrying the *optrA* and one copy of *erm*(B) could be excised from ICE*Ssu*HN38. ICE*Ssu*HN38 was able to successfully transfer into the recipient strain *S. suis* BAA by conjugation.
Conclusions: In this work, a novel *optrA*-carrying mobile genetic element, an UCS, was identified in *S. suis*. The *optrA* gene was flanked by copies of *erm*(B) and its location on the novel ICE*Ssu*HN38 will aid its horizontal dissemination.

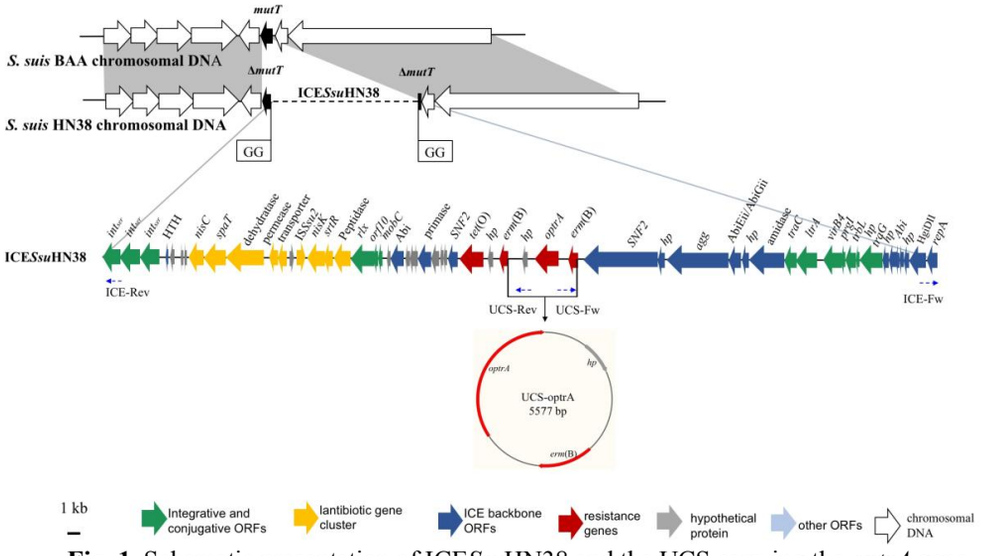

Fig. 1. Schematic presentation of ICE*Ssu*HN38 and the UCS carrying the *optrA* gene.

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P56

Identification of IS*Vlu1*-derived translocatable units containing the *optrA* and/or *fexA* genes generated by homologous or illegitimate recombination in *Lactococcus garvieae* of porcine origin

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Objectives: To investigate the presence and transfer of the oxazolidinone/phenicol resistance gene *optrA* and identify the genetic elements involved in the horizontal transfer of the gene *optrA* [1,2] in *Lactococcus garvieae*.
Methods: WGS of the *optrA*-positive isolate *L. garvieae* BN62 was performed using both Illumina HiSeq and Oxford Nanopore platforms. Antimicrobial susceptibility testing by broth microdilution followed EUCAST recommendations. The transferability of plasmid pBN62-*optrA* was evaluated by conjugation assays. Translocatable units (TUs) were detected by PCR assays followed by Sanger sequencing of the amplicons.
Results: *L. garvieae* BN62 harbored the oxazolidinone/phenicol resistance gene *optrA*, which along with *fexA* was located on the novel plasmid pBN62-optrA. Two complete and three truncated copies of IS*Vlu1* elements were found on this plasmid. The *optrA* gene was bounded by two complete IS*Vlu1* copies in the same orientation, and the *fexA* gene was bracketed by a complete and a truncated IS*Vlu1* in opposite orientations. PCR assays revealed that three different-sized IS*Vlu1*-based TUs carrying *optrA* and/or *fexA*, were formed from plasmid pBN62-optrA. The TU-*optrA* was generated by homologous recombination while TU-*fexA* and TU-*optrA*+*fexA* were the products of illegitimate recombinations. Conjugation assays confirmed the successful transfer of plasmid pBN62-optrA into *Enterococcus faecalis* JH2-2.
Conclusions: To the best of our knowledge, this is first report of *optrA*-harbouring *L. garvieae* of animal origin. Moreover, the formation of three novel IS*Vlu1*-mediated TUs underlines that IS*Vlu1* is highly active and plays an important role in the transfer of resistance genes.

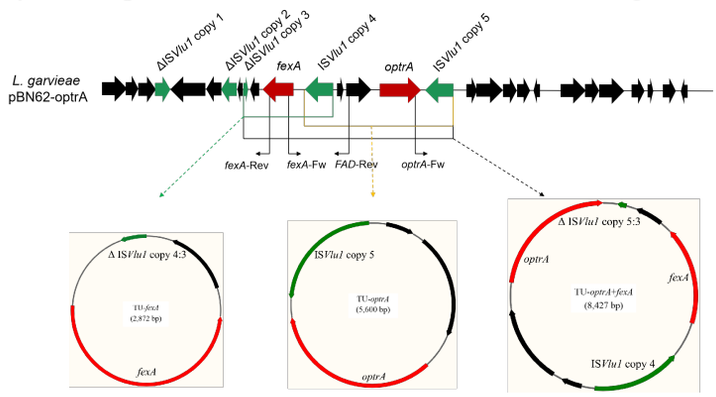

Fig. 1. Schematic presentation of three novel TUs, generated from pBN62-optrA.

References

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P57

Efflux pump's spatial properties control inhibitor binding

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Multidrug resistance is facilitated by the multidrug efflux pumps. MexB in *Pseudomonas aeruginosa* and AcrB in *Escherichia coli* are inhibited by the efflux pump inhibitor (EPI) ABI-PP, whereas MexY in *P. aeruginosa* is not. It has already been reported how ABI-PP binds to the inhibitor-binding pits of AcrB and MexB. Bulky tryptophan (Trp) was found to be the cause of the insensitivity of MexY to ABI-PP [1]. Although AcrB (Phe178Trp) is no longer inhibited by ABI-PP and MexY (Trp177Phe) is inhibited by ABI-PP, it is interesting that ABI-PP can inhibit MexB (Phe178Trp). Hence, it is unclear which changes in bulky amino acids are important for inhibitor binding in MexB. Therefore, we further examined the inhibitor binding pit of MexB to determine which Trp mutations inhibit ABI-PP binding. When positions 139, 277, 279, and 612 were changed to tryptophan, the inhibitory effect disappeared. However, the mutation at position 571 had no effect (Fig.1). Our findings suggest that mutations at various sites have a significant impact on the binding effectiveness of EPIs and that spatial factors play a role in EPI binding[2]. Finding novel inhibitors and developing new medications should take these findings into account.

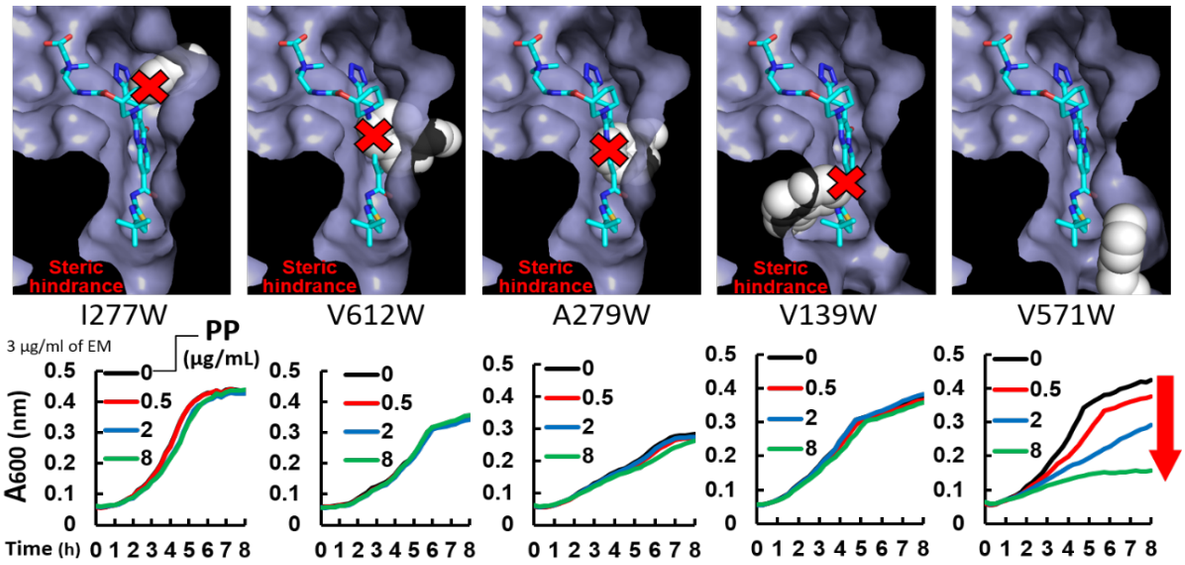

Fig. 1. Evaluation of the putative structures and inhibitory effect of ABI-PP in MexB mutants.

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P58

Investigation of the macrolide-resistance in bovine *Mannheimia haemolytica* isolates from Germany

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Mannheimia haemolytica is of considerable importance in the development of the multifactorial bovine respiratory disease (BRD). Recently, data from the German national resistance monitoring program GE*RM*-Vet showed that the number of macrolide-resistant bovine *M. haemolytica* has been slowly increasing since 2009. As bovine respiratory tract infections are often treated with macrolides, this trend may result into severely limited therapeutic options for the management of BRD. In this study, 19 macrolide-resistant bovine *M. haemolytica* isolates from GE*RM*-Vet 2008 – 2020 were investigated. Antimicrobial susceptibility testing (AST) was performed via broth microdilution according to CLSI standards (i) to confirm the macrolide resistance and (ii) to define the resistance phenotypes of the isolates. Minimal inhibitory concentration (MIC) values were obtained for the macrolides erythromycin, tilmicosin, tulathromycin, gamithromycin, and tildipirosin as well as for 22 other antimicrobial agents. All isolates either had elevated MICs or were resistant to at least one or more of the macrolides tested. In particular, isolates Mh190176, Mh191452, and Mh192916 showed resistance to tilmicosin, tulathromycin, gamithromycin, and tildipirosin, and elevated MICs for erythromycin. Furthermore, they were resistant to penicillin, ampicillin, florfenicol, and tetracycline, and had elevated MICs for gentamicin, streptomycin, neomycin, and sulfisoxazole. Based on the AST results, these three isolates were selected for whole-genome sequencing to determine the genetic basis of their multidrug-resistance (MDR) phenotypes. The DNA was prepared by phenol-chloroform extraction. Closed genomes were obtained by hybrid assembly of Illumina MiSeq and Oxford Nanopore MinION reads. Sequence analysis revealed the presence of a Tn*7406*-like integrative and conjugative element (ICE) in all three isolates containing the antimicrobial resistance genes *erm*(T), *mef*(C), *mph*(G), *floR*, *catA3*, *aac(3)-IIa, aph(3'')-Ib, aph(3')-Ia*-like, *tet*(Y)-like, and *sul2*. Isolate Mh191452 harbored an additional second copy of the *floR* gene within the Tn*7406*-like ICE. In addition, isolate Mh190176 carried a 9,226 bp plasmid with two copies of the bla_{ROB-1} gene, whereas the other two isolates each harbored a 4,614 bp plasmid (100% pairwise identity) with one bla_{ROB-1} gene. The detection of the macrolide resistance genes *erm*(T), *mef*(C), and *mph*(G) together with other resistance genes on a MDR-mediating ICE in bovine *M. haemolytica* shows that these isolates are already resistant to phenicols, penicillins, tetracyclines, and macrolides, which are regularly used for treating BRD. Due to the risk of limited therapeutic options, pathogen identification and subsequent AST is essential to ensure the efficacy of the antimicrobial agents applied to control BRD in cattle herds.

P59

Integration of transferable hybrid plasmids conferring resistance to carbapenem, colistin, and tigecycline in *Klebsiella pneumonia* isolated from house fly

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Objectives: To investigate the mechanisms for the formation of hybrid plasmids conferring resistance to carbapenem, colistin and tigecycline during the conjugation process, and to further characterize the transferability and stability of the newly formed multidrug resistant plasmids.
Methods: The locations of antimicrobial resistance genes on the plasmids were determined using S1-PFGE and Southern blot assay. The detailed genetic structures for these plasmids were depicted by a hybrid sequencing strategy using Illumina short-read and MinION long-read technology.
Results: We identified one *Klebsiella pneumoniae* isolate KP53 containing *bla*NDM-1, *mcr-8*, and *tmexCD1-toprJ1* on different plasmids. More specifically, these clinically important genes could be co-transferred through IS*26* and *ltrA* mediated plasmid fusion to clinical isolates during conjugation under colistin selection, and then the recipient strains became carbapenemor tigecycline-resistant strains. The transferability and stability of these hybrid multi-drug resistance plasmids depend on the bacterial host and the presence of antibiotics.
Conclusion: This is the first time to report the forming of hybrid plasmid conferring resistance to carbapenems, colistin, and tigecycline. The biological advantages of the fusion plasmid indicated that the fusion event presumably plays a potential role in the dissemination of AMR. Considering the strain isolated from house fly, therefore, from a “One Health” perspective, further evolution and adaptation of these hybrid plasmids may facilitate their emergence and spread, which is of great concern for clinical therapy.

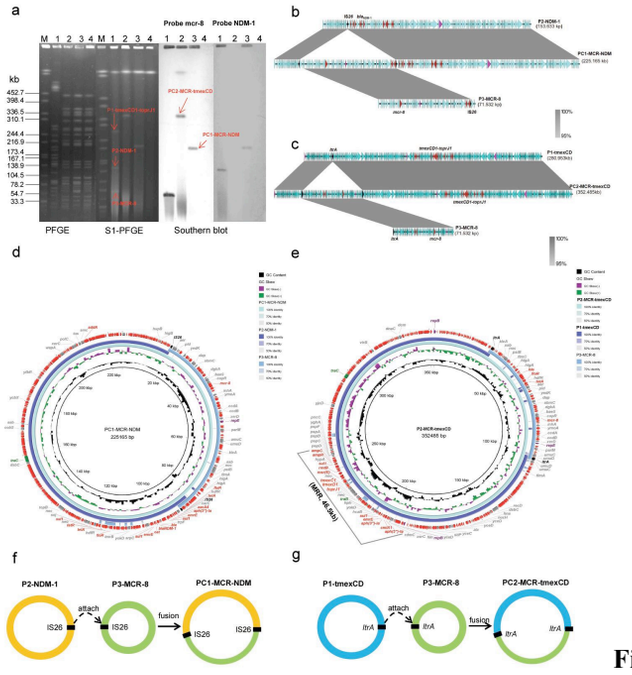

Figure 1. The integration of hybrid plasmids.

P60

Emergence of high-level tigecycline resistance due to the amplification of *tet*(A) gene variants in clinical carbapenem-resistant *Klebsiella pneumoniae*

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The emergence of various genes mediating tigecycline resistance poses a significant risk to public health safety. Since the description of *tet*(X4), which was located on a plasmid and mediated high-level resistance to tigecycline, numerous studies have been conducted on tigecycline resistance. In previous reports, *tet*(A) variants have been considered to mediate low-level resistance to tigecycline, which received much less attention in clinical research. In this study, we found a greatly high rate (62.1%, 998/1,607) of *tet*(A) variants carriage in 1,607 carbapenem-resistant *Klebsiella pneumoniae* isolates from Henan Province, China. Moreover, we confirmed that high-level tigecycline resistance could be rapidly produced by the amplification of *tet*(A) variants in these isolates. The amplification and overexpression of *tet*(A) variants were verified by the determination of gene copy numbers and qRT-PCR. Through the analysis of the raw sequencing data and the plasmid mapping depth, we found that the Δ*tnpA* homologous sequence of Tn*1721* supports the amplification of the region that harbors the *tet*(A) variants and forms a large number of repeat arrays through translocatable units (TUs). Moreover, the epidemiological analysis of *tet*(A) variant-carrying structures among 1,607 clinical carbapenem-resistant *Klebsiella pneumoniae* isolates indicated that the TU structure formed by the homologous sequence Δ*tnpA* is widely present. These results indicated that the presence of tigecycline resistance-mediating *tet*(A) variants in this clinical pathogen represents a greater health concern than initially thought and should be monitored consistently.

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P61

Are ionophore anticoccidials drivers for selection of resistance to clinical antibiotics on Norwegian poultry farms?

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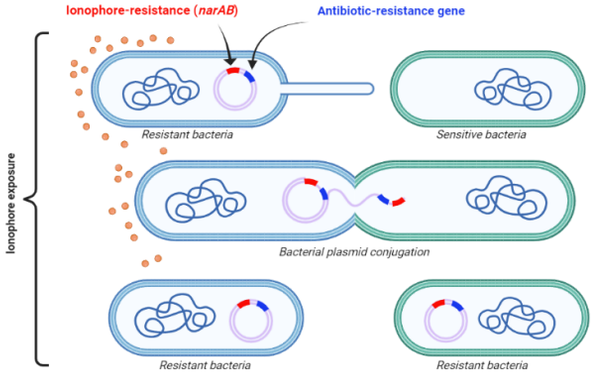
Coccidiosis, a disease caused by intestinal infection by protozoa belonging to the genus *Eimeria*, is a significant challenge to poultry health and welfare. The economic impact of coccidiosis in modern poultry production is substantial. Ionophores were introduced as anticoccidials in the 1970s, and in-feed prophylactic combinations of ionophores and synthetic chemicals are currently the most widely applied means of coccidiosis control in major poultry producing countries. Ionophores are ion carriers display both anticoccidial and antibacterial activity. Due to heavy use, widespread resistance to ionophores has been reported in both *Eimeria* and in indicator bacteria such as *Enterococcus faecium*. In 2012, it was observed that ionophore-resistance genes, later termed *narAB,* and resistance genes to last resort glycopeptide drugs (vancomycin) in *E. faecium* isolates from Swedish broilers could be co-transferred in the laboratory by bacterial conjugation [1]. Such a co-selection of ionophore and antibiotic-resistance offered an explanation for the concerning persistence of glycopeptide-resistant *E. faecium* in poultry and poultry farmers despite these glycopeptide drugs having been banned for more than two decades. The *narAB* resistance operon and its co-transfer has been found to be induced by exposure to ionophores [3]. Recently, *E. faecium* isolates from Dutch broilers have been found to carry plasmids encoding *narAB* along with resistance genes to antibiotics such as macrolides, tetracyclines and aminoglycosides [3]. Together these findings implies that the use of ionophores might drive the dissemination of resistance to medically important antibiotics in poultry. As a part of a bigger project, we have in this pilot study sequenced 20 *E. faecium* isolates from Norwegian broilers and turkeys to describe the phylogenetic distribution of AMR genes to explore whether bacteria from Norwegian poultry farms carry the potential for an ionophore-driven persistence of antibiotic resistance.



Proposed model for co-selection of ionophore- and antibiotic-resistance in poultry-associated bacteria.

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P62

Identification of the novel Macrolide-Lincosamide-Streptogramin B resistance gene *erm*(54) in a porcine LA-MRSA ST398

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Background and objectives: Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) can represent a health risk for humans, especially for those having frequent contact to livestock [1]. LA-MRSA are mainly associated with sequence type (ST) 398 in many parts of the world and often multiresistant to antimicrobial agents [1]. Here, 178 porcine LA-MRSA isolated in Germany from 2007 - 2019 were investigated for new antimicrobial resistance genes.
Methodology: Whole-genome sequencing on Illumina MiSeq and PacBio Sequel II platforms was followed by hybrid assembly and sequence analysis. Plasmid pHKS3860 was transferred into *S. aureus* RN4220 via electrotransformation. Antimicrobial susceptibility testing via broth microdilution and agar disc diffusion was performed according to CLSI standards [2] to confirm the functionality of *erm*(54). An *erm*(54)-specific PCR assay was developed and applied to 30 macrolide-resistant staphylococcal isolates, which harbored next-related *erm* genes.
Results: A novel Macrolide-Lincosamide-Streptogramin B (MLS_B) resistance gene, *erm*(54), was detected on the non-conjugative plasmid pHKS3860 of 36,929 bp in a porcine LA-MRSA ST398. The gene encoded a 23S rRNA methylase of 245 amino acids (aa) that was next-related to Erm(B) (72%). Moreover, *erm*(54) was expressed constitutively. A complex regulatory region composed of a small reading frame for a 30 aa protein and seven pairs of inverted repeats, which can form varying mRNA secondary structures, was detected upstream of *erm*(54). The transferred *erm*(54) caused a distinct increase in the minimal inhibitory concentrations of MLS_B antibiotics in *S. aureus* RN4220. The new PCR assay detected *erm*(54) in the original strain and the transformants carrying pHKS3860, but none of the next-related *erm* genes available to us. Copper, mercury and cadmium resistance genes as well as an *ica* cluster for biofilm formation were also found on plasmid pHKS3860.
Conclusion: The new transferable and functionally active MLS_B resistance gene *erm*(54) was identified in a porcine LA-MRSA ST398 isolate from Germany. The co-location of *erm*(54) on a plasmid with heavy metal resistance genes may increase the risk for co-selection under selection pressure imposed by heavy metals in animal feed or the environment. Due to this possibility of co-selection and the zoonotic potential, *erm*(54)-carrying isolates might pose a public health threat in the future.

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P63

Allicin served as a New Delhi metallo-β-lactamase inhibitor：a new strategy to overcome superbugs

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The emergence and spread of carbapenem-resistant Enterobacteriaceae (CRE), mediated by metallo-β-lactamases (MBLs) like NDM, is a global public health problem. It is becoming a new consensus repurposing approved drugs or natural compounds as potential MBLs inhibitor to restore the efficacy of carbapenem. Previous studies have demonstrated that allicin, the main effective ingredient in garlic, has many pharmacological effects including antibacterial, anti-inflammatory, antiparasitic, anti-tumor, and treatment of cardiovascular and cerebrovascular diseases and been used in the treatment and prevention of a wide variety of ailments. In this study, we focus on investigating the effect of allicin on CRE as well as its inhibitory activity on NDM enzyme. Shown by the following figure, allicin displayed significant antibacterial activity against NDM-carrying *E. coli* strains with MIC values from 16-64 μg mL^{-1} and presented excellent synergistic activity with meropenem (FICI=0.357). In addition, we identified that allicin is an effective inhibitor of the NDM enzyme with 50% inhibiting concentration (IC50) was 18.63 ± 1.39 μM. The equilibrium dissociation constant (Kd) to NDM was 0.14 ± 0.087 μM, which was determined by microscale thermophoresis. AutoDocking analysis indicated that allicin inactivates NDM by binding to the active site Cys208. And the zinc sensitivity experiments showed that the binding of allicin to NDM resulted in the release of Zn(II). Finally, a mouse leg muscle model infected with NDM-carrying *E. coli* proved that the combination of allicin and meropenem significantly reduced the bacterial load. In conclusion, our findings demonstrate that the combination of allicin and meropenem, a carbapenem antibiotic, has therapeutic potential to address the clinical challenge of MBL-positive CRE strains.

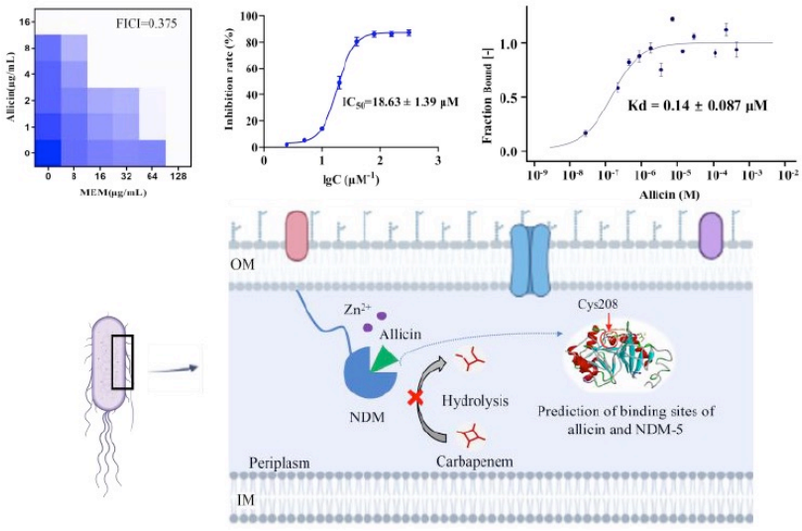

Fig. 1. Allicin served as a NDM inhibitor.

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P64

The AcrAB Efflux Pump Confers Intrinsic Multidrug Resistance and Self-Protection against Stilbenes in *Photorhabdus laumondii*

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The global shortage of new antimicrobials brought us to antimicrobial resistance which has emerged to last-resort antibiotics. The Resistance-nodulation-division (RND)-type AcrAB-TolC efflux pump contributes to multidrug resistance in Gram-negative bacteria. Recently, the bacterium *Photorhabdus laumondii* TT01 has emerged as a goldmine for novel anti-infective drug discovery. Outside plants, *Photorhabdus* is the only Gram-negative known to produce stilbene-derivatives including 3,5-dihydroxy-4-ethyl-trans-stilbene and 3,5-dihydroxy-4-isopropyl-trans-stilbene (IPS). IPS is a bioactive polyketide which received considerable attention, mainly because of its antimicrobial properties, and is currently in phase III clinical trials as a topical treatment for psoriasis. To date, little is known about how *Photorhabdus* survives in the presence of stilbenes. We combined genetic and biochemical approaches to assess whether AcrAB efflux pump exports stilbenes in *P. laumondii*. We demonstrated that the wild type (WT) exerts an antagonistic activity against its derivative Δ*acrA* mutant, and that is able to outcompete it in a dual-strain co-culture assay [1, 2]. The Δ*acrA* mutant also showed high sensitivity to 3,5-dihydroxy-4-ethyl-trans-stilbene and IPS as well as decreased IPS concentrations in its supernatant comparing to the WT [2]. We report here a mechanism of self-resistance against stilbene derivatives of *P. laumondii* TT01, which enables these bacteria to survive under high concentrations of stilbenes by extruding them out via the AcrAB efflux pump [2]. Our findings emphasize the poly-specificity and versatility of AcrAB to export diverse native antimicrobial compounds in Gram-negative bacterial species and may represent a promising target feature to improve the control of resistance to innovative antibiotics.

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Session 4 - Understanding the connection of antimicrobial resistance between Animals and Humans

P65

The role of sub-inhibitory antibiotic pollution on MGEs in the environment

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Antibiotic residues resulting from anthropogenic activities are released to the environment and often at concentrations that are under the bacterial growth inhibition threshold. Sub-inhibitory concentrations of antibiotics, despite being too low to inhibit overall bacterial growth in environmental communities, might impose a selective pressure on some community members and induce a response in environmental bacteria. Consequently, the presence of antibiotic residues at sub-inhibitory concentrations in the environment could lead to the mobilization of antibiotic resistance genes (ARGs) in mobile genetic elements (MGEs) and the dissemination of these MGEs in environmental settings and to the human microbiome through horizontal gene transfer (HGT). Although increasing evidence points to a potential risk for human health associated to environmental sub-inhibitory antibiotic pollution, the magnitude of this risk still needs to be elucidated. Our research addressed the role of sub-inhibitory concentrations of antibiotics on the mobilization of environmental ARGs into MGEs such as class 1 integrons and plasmids, and the resulting dissemination potential of these MGEs in the environment and to the human microbiome. We used a combination of culture-based and metagenomics approaches to evaluate the selective potential of sub-inhibitory concentrations of a range of antibiotics (e.g., gentamicin, ciprofloxacin) with or without heavy metals and microplastics on MGEs and their associated ARGs. We evaluated the influence on the metagenomes of the river microbial community in order to delineate possible mechanisms underlying the response of these MGEs to sub-inhibitory pollution. Antibiotics at sub-inhibitory concentrations increased the mobilization of antibiotic resistance genes in class 1 integrons and the dissemination potential of these genes. Our research shows molecular evidence of the impact of antibiotics at sub-inhibitory concentrations on environmental MGEs and the mechanisms underlying the molecular response to sub-inhibitory antibiotic pollution.

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Investigative genomics on highly antibiotic resistant *Pseudomonas* isolated from rainbow trout (*Oncorhynchus mykiss*)

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Open aquaculture systems are farming areas with strong relationships between food-producing fishes and their aquatic environment. Aquatic animals are exposed to several physico-chemical variations and to human or terrestrial animal discharges with chemical and microbiological contaminants. Fishes host autochtonous bacteria which can encounter allochtonous ones brought by the river inflow in farms. *Pseudomonas* is a bacterial genus that is ubiquitous in many environments and often isolated from fish such as rainbow trout and their environment[1,2]. We previously identified *Pseudomonas* isolates collected on rainbow trout fillets from two different farms, eight of which showing high minimum inhibitory concentrations (MICs) to one or several antibiotics[3]. Seven out of eight isolates showed multiple high MICs to oxytetracycline (64 to 256 µg.mL^{-1}), florfenicol (512 to >1,024 µg.mL^{-1}), colistin (512 to >1,024 µg.mL^{-1}) and trimethoprim/sulfamethoxazole (128/2432 to 512/9728 µg.mL^{-1}).

In this study, we focused on genome architecture of these eight isolates with high MICs to establish relationship between phenotype and genotype, and studied the presence of mobile genetic elements (MGEs) to better understand if these genes can be transferred horizontally. After DNA isolation (DNeasy Blood & Tissue Kit by Qiagen and Wizard® Genomic DNA Purification Kit by Promega) of each *Pseudomonas* isolate, genomic DNA was sequenced using Illumina (short reads) and Oxford Nanopore (long reads) technologies. Hybrid genomes assembly were performed using UniCycler then scaffolding of all assemblies at contig levels were carried out and compared to a closely related genome used as a reference sequence (*P. fluorescens* SBW25). Subsequent searches of antibiotic resistance genes have been done using the Resistance Gene Identifier tool of the Comprehensive Antibiotic Resistance Database (CARD) and completed with the ResFinder tool. MGEs as insertion sequences, transposons, plasmids have been investigated using ISfinder and the plasmid database PLSDB when applicable. Prophage sequences were also searched using PHASTER.

The sequenced genomes showed a total length ranging between 6Mbp and 6.5Mbp. In-depth analyses of these genomes established that most of high MICs could be related to at least one known antibiotic resistance gene with *tetY* for oxytetracycline, *floR* for florfenicol and *sul2* for trimethoprim/sulfamethoxazole. No *mcr* gene was detected for the two isolates with high MICs to colistin. Additional genes related to unassessed phenotype resistance have been identified such as *AbaQ* assuming to encode for an efflux pump mediating quinolone resistance or *aph(6)-Id* and *aph*(3')-Ib already known for coding aminoglycoside phosphotransferases. A wide range of insertion sequences and transposons were also detected in all isolates such as IS66, IS5, Tn3 or Tn5 families. Every *Pseudomonas* isolate comprised prophage sequences in several regions of their genomes with intact or incomplete match with common phages. Our results highlight potential genetic bases for atypical phenotypes found in *Pseudomonas* hosted by rainbow trout. This study reinforces the interest to better study bacterial whole genomes to provide new elements for understanding the role of horizontal gene transfer in their architecture but also the spreading of antibiotic resistance.

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Administration of oxytetracycline and resistance selection in the different digestive segments: determination of selective minimum concentrations and impact on the microbiota and resistome in pigs

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After oral administration, a fraction of antibiotics binds to the digestive matrix. Nevertheless, the free or active concentrations exert a pressure of selection on the microbiota, favouring resistance selection. Therefore, the first objective of this study was to determine the minimum selective concentration (MSC) of oxytetracycline (OTC); the MSC being the lowest concentration of an antibiotic conferring a competitive advantage to a resistant strain upon an isogenic susceptible strain. The second objective was to characterise the impact of OTC administration on the microbiota and resistome in the different digestive segments of pigs.

Co-cultures of an OTC-susceptible *Escherichia coli* strain and a resistant isogenic strain were performed in the presence of different concentrations of OTC in Muller-Hinton broth (MHB) and sterilised intestinal contents (SIC) of the jejunum, cecum and rectum of pigs. These strains were counted at different times, permitting to calculate the selection coefficient at each concentration of OTC and, by extrapolation, the MSC [1]. Subsequently, a therapeutic dose of OTC (20 mg/kg bw) was administered orally to eight piglets. The contents of the digestive segments were harvested 6 (n = 4) and 24 hours (n = 4) after treatment. The control group consisted of four untreated animals. Tetracycline resistance genes were quantified by qPCR. Microbial diversity was assessed by sequencing the V3-V4 region of the gene encoding 16S rRNA. Finally, OTC was quantified by UPLC-UV to determine the total concentrations in the different digestive segments.

MSC was 0.025 µg/mL in BMH and increased to 0.26, 0.72, and 2.4 µg/mL in the jejunum, cecum, and rectum, respectively. The binding between antibiotics and constituents of the digestive matrix may have influenced the free (active) antibiotic fraction, this bond being stronger in the distal digestive tract [2]. In addition, the interaction between bacteria and constituents of the digestive matrix may have influenced their sensibility to antibiotics [3]. Along the digestive tract, the taxonomic composition of the microbiota changed, and the abundance of resistance genes increased, but the antibiotic treatment did not impact the microbiota. Finally, 6 h after administration, OTC concentration was 49.7, 65.9 and 5.7 µg/g, and 12 h after administration the concentrations were >LOQ, 18.6 and 97.9 µg/g in the contents of jejunum, cecum and rectum, respectively. We will determine the active OTC concentrations using bactericidal curves produced in MHB and SIC.

In conclusion, this study provides new data for the risk assessment of antibiotic resistance in the digestive environment. In addition, we plan to improve our *in vitro* model to consider the effect of gut microbiota on MSC. Finally, the developed methodology could also be applied to the risk assessment of other antibiotics or other matrices (e.g. environmental samples).

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Development of a *Salmonella enterica* Plasmid Transfer Gene Database and Associated Analysis Tools

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Antimicrobial resistant (AMR) enteric pathogens, including *Salmonella*, has become a significant concern due to potential treatment failures. Often, AMR genes are encoded on transmissible plasmids and the conjugal transfer of resistance plasmids can lead to the dissemination of AMR among bacterial populations. Type IV secretion systems (T4SSs), which are integral parts of the conjugation process in enteric bacteria, are encoded on the transfer (*tra*) regions of plasmids. Better understanding of the diversity and functionality of the T4SS genes that can facilitate the development strategies to control and prevent spreading of temergence of multidrug-resistant pathogens. To facilitate the analyses of these transfer-associated genes, the current project was undertaken to identify the transfer associated genes that are associated with conjugal secretion systems in key groups of AMR plasmids. The information of these transfer-associated genes was extracted from GenBank and incorporated into a Plasmid Transfer Gene Database. After the database set up, two comparison tools were developed. One of the tools named "the Plasmid Transfer Factor Assessment" is used to predict the transfer genes present in the specific plasmid location and their sequence similarity to the reference genes. The other tool was named the "Plasmid Transfer Factor Comparison tool", which can be used to facilitate the comparison transfer genes of plasmids from multiple sequence files. To assess the databases, plasmid and whole genome sequencing (WGS) data identified from GenBank and previous WGS experiments in our lab were extracted and used to assess the tools. Overall, when plasmids were identified with PlasmidFinder, their T4SS-associated genes were typically identified with the database tools. In some cases, there were significant overlaps such as with genes from IncBO and IncK plasmids, thus the database was updated to combine the genes from these plasmid types into a renamed IncB/O/K group. The current updated database contains 337 genes representing the T4SSs from 13 different plasmid groups. The plasmid transfer database and tools proved very useful technique for evaluating the different plasmid types and their T4SSs. The ability to predict all T4SS genes that are present can improve our understanding how conjugative plasmids contribute to the dissemination of antimicrobial resistance genes.

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Screening for CPE in livestock in the Netherlands using multiplex real-time PCR

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Background: Carbapenemase-producing *Enterobacteriacea* (CPE) are an increasing problem worldwide. As a consequence monitoring of CPE in livestock and meat became mandatory in 2021 after the new European legislation on antimicrobial resistance monitoring in livestock and meat came into force. The prescribed method for detection of CPE is selective culturing, but this method has its limitations [1]. For this reason a reliable and cost-effective method for rapid and sensitive screening of CPE in fecal samples is desired. In this study a set of two multiplex real-time PCR assays was developed.

Method: Previously published primers were combined with in-house designed primers to set up the multiplex real-time PCR assays using SybrGreen chemistry. The first multiplex real-time PCR covers the most predominant carbapenemase genes *bla*$_{NDM}$, *bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{OXA}$. The second multiplex real-time PCR covers the more rarely detected genes *bla*$_{IMI}$, *bla*$_{FRI}$ and *bla*$_{FLC}$ earlier reported in *Enterobacter cloacae* complex isolates from imported fish and shrimps [2]. This set of PCR's is completed with an amplification control PCR to test for inhibitory factors in the DNA. The scope of the first PCR was focused on the gene variants reported in European livestock [3] (table 1). Primers are selected for each gene group to amplify all gene variants listed in table 1. Subsequent sequencing of the amplicons is necessary to determine the specific gene variants.

Table 1 Carbapenemase gene variants found in European livestock

Target	NDM	KPC	VIM	IMP	OXA
Gene variants	NDM-1 NDM-4 NDM-5 NDM-7 NDM-9 NDM-17	Not specified	VIM-1	IMP-1 IMP-2 IMP-27	OXA-48/OXA-48-like OXA-58 OXA-181

For CPE monitoring of livestock in the Netherlands pools of five selective enrichment samples are tested with the described set of PCR's. When a pool is tested positive with a PCR, the individual samples of the pool will be tested. Additionally, carbapenem resistant isolates grown from positive enrichment samples are tested with the PCR-set. The presence of carbapenem genes in CPE-suspected isolates is confirmed by sequencing. To date, no CPE were detected in faecal samples of livestock in the Netherlands.

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Onsite and rapid detection of tigecycline-resistant hypervirulent *Klebsiella pneumoniae* using CRISPR/Cas12a based assay

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Tigecycline-resistant hypervirulent *Klebsiella pneumoniae* (HvKP) is a notorious pathogen that poses a significant threat to public health, as it could cause severe infections with high morbidity and mortality among young and healthy individuals. Currently, a positive string test has been primarily used to identify HvKP isolates. However, this method with an accuracy of 90% is more suitable for hypermucoviscous phenotype. An accurate and rapid assay to identify tigecycline-resistant HvKP infections is needed for optimal clinical care and infection control efforts. Therefore, in the present study, CRISPR/Cas12a based method was developed for onsite detection of tigecycline-resistant HvKP based on the virulence biomarkers *iucA*, *iroB*, *peg-344*, *rmpA* and *rmpA2*, and the resistance gene *tet*(A). Upon optimization, the CRISPR/Cas12a based method detected 1 cfu/reaction of tigecycline-resistant HvKP with high accuracy and specificity, and the whole procedure took 60 min including DNA rapid extraction. Moreover, the whole process can be performed with a portable block heater, and the detection results can be observed with the naked eye. The developed CRISPR/Cas12a assay could be used as a diagnostic tool with onsite, portable, rapid, and simple for the practical identification of tigecycline-resistant HvKP infections.

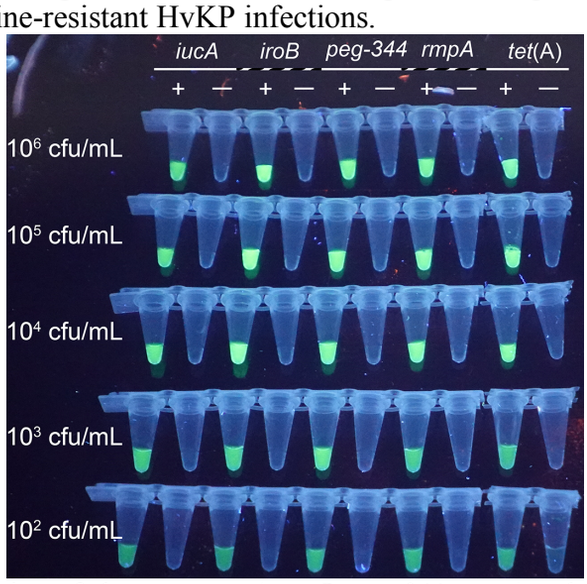

Fig. 1. Simultaneous detection of the genes *iucA*, *iroB*, *peg-344*, *rmpA* and *tet*(A) using the developed CRISPR/Cas12a method. Strains with 10^6, 10^5, 10^4, 10^3 and 10^2 cfu/mL were used in this study. A positive symbol (+) indicates that the extracted DNA was added as the template, while a negative symbol (–) implies that nuclease-free water was used as the negative control.

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Conifer needles as a passive air sampler for antibiotic resistance genes (ARGs) detection emitted during pig manure spreading

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Bacterial antibiotic resistance was identified worldwide as an urgent global public health threat. Antibiotic resistance genes (ARGs) can be acquired and/or transferred by bacteria through different mechanisms. Due to the heavy usage of antibiotics in agricultural activity, ARGs are abundant in manure and can potentially be dispersed in the environment over significant distances by spreading activities on fields. ARGs propagation should be studied to better assess the antibioresistance risk. Biomonitoring of air is generally performed using active samplers. However, these devices are expensive, bulky and recover air for a relatively short time window. Passive samplers, such as conifer needles, could be used as an alternative for air monitoring. This project aims to assess the ARGs accumulation on conifer needles compared to active high flow sampling during pig manure field spreading. Four spreading activities of pig slurry were conducted on an experimental farm. Air samples were taken with two high-flow samplers, during swine manure spreading. Downwind (3 distances) and upwind samples were collected. Conifer needles, from trees located downwind of the sampling site were sampled before and after (24h) spreading activities. Conifer needles processing has been achieved according to George et al. [1].Total bacteria concentration was quantified by quantitative PCR (qPCR) for the pig manure, the conifer needles, and the air samples. Chloroplasts were quantified in conifer needles (rcbL). One fecal indicator and one pig DNA indicator (phage vB_AviM_AVP and *Sus domesticus* cytochrome b gene) were assessed in conifer needles and air samples bioaerosol source confirmation. Manure samples were tested for the presence of 40 different ARGs [2]. Amongst the genes tested, 28 were detected and are associated with resistance to sulfonamides (2), tetracyclines (10), erythromycin (5), β-lactams (2), quinolones (2), aminoglycosides (3), and colistin (1). Mobile genetic elements (3) were also detected. These positive genes were used to analyse the conifer needles and the air samples with a high-throughput qPCR Smartchip platform (Takara). For both conifer needles and air samples, the most frequently detected ARGs were in accordance with what is observed in manure samples: tetracyclin (tet) and erythromycin (erm). The genes with the highest frequency in conifer needles were tetW and erm35 (72% and 14% respectively). In air samples tetW and ermB (44% and 33% respectively). Manure spreading from animal farms lead to dissemination of ARGs in the environment and can potentially represent a threat for human and animal health. Conifer needles could be a simple and affordable long-term sentinels to monitor ARGs dispersion through bioaerosols.

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MyMIC: a network for standardization of diagnostics, antimicrobial susceptibility testing and clinical interpretation in animal mycoplasmas

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Mycoplasma is a bacterial genus that is often disregarded although it includes species that are of importance for both human and animal medicine. In animals, some species are major pathogens causing various diseases and significant economic losses in avian, porcine and ruminants sectors. In the absence of efficient vaccines, their control relies mainly on chemotherapy and thus mycoplasmas contribute largely to antibiotic use and hence to the general rise of resistance. Because of their intrinsic resistance to the broadly used β-lactams family and the increasing number of acquired resistances to other antimicrobial families [1,2], mycoplasmas must be subjected to an improved antimicrobial resistance surveillance. However, standard procedures, used for classic bacteria to assess the efficacy of antibiotics by *in vitro* antimicrobial susceptibility testing (AST), such as disk diffusion method, cannot be used for mycoplasmas, because of their fastidious growth and requirement for complex medium. Moreover, the lack of guidelines, quality control (QC) strains, clinical breakpoints or epidemiological cut-off (ECOFF) values for livestock mycoplasmas makes comparisons between studies and interpretation of results difficult or impossible. MyMIC is one of the six new collaborative networks funded by the Joint Programming Initiative on Antimicrobial Resistance (Diagnostics and Surveillance Networks – JPIAMR). It includes 22 laboratories from 18 countries working on animal *Mycoplasma* diagnostics and AST. The objective of this network is to make an inventory of the different methods used by laboratories for identification, culturing, determination of minimum inhibitory concentrations (MIC) per animal and *Mycoplasma* species, and detection of resistance mechanisms by molecular or genomic tools. The MIC values of the main families of antimicrobials used against some key pathogenic *Mycoplasma* species, obtained by identical or comparable methods, will also be collected and aggregated to determine if this data can be used to define first tentative ECOFF values. This network will lead to the drafting of guidelines for a standardized culturing, identification and determination of MIC for livestock *Mycoplasma* species. Moreover, it could be the start of new wet-lab research proposals for additional work to undertake in order to define QC strains, ECOFFs and clinical breakpoints.

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Application of MALDI-TOF MS for detection of carbapenem resistance in bacteria

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Background and objectives: Over the last few years, MALDI-TOF MS has been explored as a rapid antimicrobial resistance detection tool. This application is based on the fact that MALDI-TOF MS can easily monitor changes in antibiotic structure that can be related to bacterial resistance. In this study, we investigate specific mass shifts caused by hydrolysis in the β-lactam ring in three selected carbapenems, i.e., meropenem, ertapenem, and imipenem.
Materials and methods: Based on the MIC values 13 *Escherichia coli* (8 sensitive, 5 resistant to carbapenems) and 7 *Salmonella* spp. (4 sensitive, 3 resistant) strains were selected for the study. Fresh overnight nutrient agar cultures (Biomaxima) were employed for the MALDI-TOF MS analysis. Samples were prepared in duplicate, positive, negative, and antibiotic control was added to the study. An inoculation loop (1 µl) of bacterial culture was suspended in 20 µl of antimicrobial solutions (concentration 0,5 mg/ml) and incubated (37°C, 500 rpm, 30 min). Subsequently, the cultures were centrifuged (2 min, 13,000 g, room temperature), and 1 µl of the supernatant was spotted onto the MALDI target plate covered by 1 µl of HCCA matrix and used for mass spectrometry analysis. Peaks were manually selected using the flexAnalysis 3.4 software (Bruker Daltonik). Only peaks belonging to the corresponding antibiotic drug and the respective degradation products were labeled.
Results: In the case of meropenem and imipenem, several peaks were present in spectra obtained from sensitive and resistant strains; however, it was not possible to distinguish characteristic profiles. On the contrary, the pattern observed for ertapenem allowed for the differentiation of sensitive and resistant strains. Carbapenem-sensitive strain revealed 498.7 Da molecular peak corresponding to the single sodium adduct of ertapenem. Furthermore, several adduct peaks were found, i.e., 514.7 Da, 520.7 Da, and 536.6 Da, representing [M+K]+, [M+2Na]+, and [M+Na+K]+ ertapenem derivatives, respectively. In the spectra of carbapenem-resistant strains, the presence of a 450 Da peak was found, which corresponds to the hydrolysed, decarboxylated form of ertapenem. The obtained differentiation of ertapenem-resistant and sensitive strains was congruent with MIC values. MALDI-TOF MS has shown its ability to correctly identify resistance to ertapenem in 30 min.
Conclusion: The most reliable results were obtained for ertapenem i.e., it was possible to clearly distinguish between susceptible and resistant profiles. The method is promising, despite the fact that there are some difficulties in the interpretation of the spectra caused by the formation of sodium and potassium adducts as well as cluster ions of the tested molecules.

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The respiratory microbiome of piglets displays temporal development and harbours strains with putative probiotic use against common respiratory pathogens.

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Introduction Bacterial pathogens residing in the porcine respiratory tract undermine animal health and welfare, and some are a zoonotic risk. We investigated the development of the porcine nasal microbiome to identify and isolate bacterial species negatively associated with common porcine respiratory pathogens. These isolated species have potential for *in vivo* pathogen reduction strategies.
Methods Nasal swabs were obtained from 252 piglets, 7 per litter from 36 sows, from 9 farms in 3 countries (Germany, Ireland, the Netherlands), at16 sampling moments from birth till week 10 (n = 4032). DNA was isolated and duplicate samples were stored at -80C for bacterial isolation. Using V3-V4 16S rRNA (n = 813) and *tuf* (n = 538) gene sequencing data of overlapping samples, correlation analysis was performed to identify microbial associations using SparCC. Additionally, sequencing data was supplemented with *Staphylococcus aureus* specific qPCR data. Species negatively associated with *S. aureus* were identified using mixed model and rmcorr. They were isolated, followed by *in vitro* (phenotypical antimicrobial resistance testing) and *in silico* (whole genome sequencing, taxonomy, antimicrobial resistance genes, virulence factors) safety screening, following EFSA guidance.
Results Amplicon sequencing allowed us to describe the developing piglet nasal microbiome. From the microbiome data, network analysis identified 26 unique taxa negatively associated with pathogens as *Bordetella sp., Glaesserella parasuis, Mycoplasma hyorhinis, Pasteurella multocida, S. aureus,* and *Streptococcus suis*. The found taxa's negative association was consistent over the three countries. qPCR supplementation to amplicon sequencing data identified a further 54 species negatively associated with *S. aureus*. Literature screening of the identified species/taxa resulted in 15 probiotic candidates against *S. aureus.* The candidates were consisting of lactic acid bacteria (LAB) and species closely related to the *Staphylococcus* genus. An isolation effort, followed by *in vitro* and *in silico* safety and efficacy studies, yielded three suitable LAB strains, meeting the Qualified Presumption of Safety (QPS) status from EFSA. These species were subsequently utilized in an animal trial.
Conclusion The porcine nasal microbiome displays a distinct development in time. This development is varying between individual piglets but on average consistent between the studied countries. Further investigation of the developing porcine nasal microbiome resulted in probiotic candidates aimed at pathogen reduction strategies. Three probiotic LAB strains against *S. aureus* are currently under study *in vivo.*

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Does hyperpersistence influence the evolution of *Escherichia coli* toward fluoroquinolone resistance ?

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Persistence is a phenomenon in which a subpopulation of bacteria, known as "persisters," enters a dormant state, enabling them to transiently survive bactericidal antibiotic concentrations. Hyperpersistent strains produce a much higher proportion of persisters in their populations, which can compromise infection treatments. Furthermore, previous studies have shown that hyperpersistence can promote the acquisition of "classical" antibiotic resistance, i.e., the ability of bacteria to grow in the presence of antibiotics.

Previous studies on persisters were conducted using static antibiotic concentrations. However, in this study, we aimed to test whether hyperpersistence would promote the acquisition of fluoroquinolone resistance in *Escherichia. coli* under fluctuating antibiotic concentrations that better mimic real-world therapy. To achieve this, bacteria were grown in a dynamic in vitro system where they received fresh medium continuously, and ciprofloxacin twice a day to reach a peak concentration of 1 µg/mL. We examined the evolution of populations of three isogenic *E. coli* strains that displayed either a susceptible phenotype (S), hyperpersistence (H, *gyrB* L422P mutation), or low-level resistance to fluoroquinolones (R, *gyrB* S464Y mutation), under exposure to fluctuating ciprofloxacin concentrations over a 10-day period. We monitored bacterial populations as well as their level of susceptibility to ciprofloxacin during the experiment.

Regardless of their initial phenotypes, the three *E. coli* strains showed adaptation of their populations, allowing them to better survive the repeated ciprofloxacin doses over the 10-day treatment. Similar levels of resistance to ciprofloxacin and survival rates were observed in the bacterial populations and clones derived from the three parental strains. Additional *gyrA* quinolone resistance mutations were identified in clones derived from the S and R parental strains, but not from the hyperpersistent strain.

In conclusion, in our experimental conditions, hyperpersistence did not appear to promote the evolution of bacteria toward high ciprofloxacin resistance levels, but may have influenced the mechanism of adaptation to the 10-days treatment.

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P76

Horizontal gene transfer between bacteria inside the intestinal microbiota of the nematode *Caenorhabditis elegans*

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Bacteria have the remarkable property of being able to adapt quickly and continuously to their environment, thanks to their ability to exchange and combine mobile genetic elements (MGE). These MGEs, which notably include conjugative plasmids and transposons, are the main carriers of antibiotic resistance genes. They thus promote the emergence of multi-resistant bacteria, a serious threat currently weighing on global health.

So far, little is known about the propagation of these MGEs within complex bacterial communities. Here, we aim to study the spatiotemporal dynamics of MGE exchanges using the nematode *Caenorhabditis elegans* as a simple experimental model of a gastrointestinal system harboring controlled bacterial communities. The natural microbiota of *C. elegans* has only recently been described, opening up the possibility of using this simple yet powerful model system in studies of the host microbiota *(Zhang et al., 2017)*. The composition of the microbiota, which is composed of a high diversity of bacterial species, the dominant group comprising several ϒ-proteobacteria (*Enterobacteriaceae, Pseudomonaceae and Xanthomonodaceae*), was shown to tune *C. elegans* health *(Haçariz et al., 2021)* and pathogenesis *(Radeke and Herman, 2021)*. *C. elegans* is also a model of choice for our study for technical reasons, such as ease of culture, short life cycle, selfing hermaphroditism allowing maintenance of homozygous cultures, the possibility to generate large numbers of nearly genetically identical individuals with identical life histories, and importantly transparency under light microscopy allowing direct visualization of bacterial cells *in vivo*. We use the CeMBio bacteria as a simplified natural *C. elegans* microbiota *(Dirksen et al., 2020)*. This includes a dozen of environmental bacterial strains from several classes (α-, β- and γ-proteobacteria and Bacteroidetes) that have been selected from meta-analyses of the natural microbiome of the nematode.

We first focus on the establishment of the CeMBio community inside *C.elegans'* intestine, which is poorly described (i.e. localization, proportion and stability along time of the different bacterial species of the microbiota in the intestine). We are also constructing the tools that allow us to analyze and compare the fate of several broad host range conjugative plasmids and transposons from several families within the CeMbio community, both *in vitro* on agar plates or in liquid cultures and *in vivo*, in the gut of *C. elegans* intestine.

By combining several approaches including bacterial genetics, fluorescence microscopy, and bio-informatics, our data could provide a better understanding of how MGEs propagate in complex ecosystems such as microbiota and thus contribute to the research for ways to limit the emergence of multi-resistant bacteria.

P77

Antimicrobial residues and the minimal selective concentration

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Introduction
The global emergence of antimicrobial resistance (AMR) is a cause for concern. Use of antimicrobials contributes to AMR. A particular problem in the use of antimicrobials in livestock is that antimicrobial residues are excreted in the same environment in which the animals are kept. It is believed that after the application of antimicrobials, they are partly excreted through urine and feces and thereafter quickly degrade. However, some antimicrobials proved to be very persistent. In this study, we examined whether persistent antimicrobial compounds remain longer in the animal environment and if the remaining concentrations still have selective properties by determining the minimal selective concentration (MSC).

Methods
The MSC was determined through fecal fermentation studies. An animal trial was conducted to determine the persistence of the three antimicrobials over time. Four groups of broilers were divided in three subgroups (n=12). Groups were left untreated (control) or were treated with amoxicillin (non-persistent), doxycycline or enrofloxacin (persistent). We determined the resistome by shotgun metagenomics using Illumina sequencing and did an phenotypical resistance analysis of *Escherichia coli* isolates. Antimicrobials were extracted from the fecal samples and analysed by LC-MS/MS.

Results
After treatment, persistent antimicrobials (doxycycline and enrofloxacin) had concentrations equal to or higher than the MSC established in the fermentation study, in contrast to the amoxicillin treatment. The doxycycline treatment showed an increase in phenotypically resistance and an increase in resistance genes in the resistome. In contrast, the amoxicillin treatment only showed an increase in the resistance genes directly after treatment. Enrofloxacin treatment resulted in merely enrofloxacin resistant *E. coli* and the resistance genes in the resistome increased during the experiment.

Conclusions
Persistent antimicrobials remain longer in the animal environment at selective concentrations. Our findings suggest that persistency of antimicrobials should be taken into consideration in the assessment of priority classification of antimicrobials.

P78

Development of first quality control ranges for biocide susceptibility testing

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Introduction: Quality control (QC) is important for laboratory tests. Therefore, the aim of this study was to develop QC ranges for biocide susceptibility testing (BST).
Materials and Methods: Four reference strains, namely *Staphylococcus aureus* ATCC® 6538, *Enterococcus hirae* ATCC® 10541, *Escherichia coli* ATCC® 10536 and *Pseudomonas aeruginosa* ATCC® 15442, were chosen as quality control strains. During an interlaboratory trial eleven laboratories tested these four strains and four biocides, namely benzalkonium chloride, chlorhexidine, octenidine and polyhexanide ten times using broth microdilution with three lots of tryptic soy broth. The RangeFinder software was used for data analysis.

Results: Overall, comparable results were observed by the eleven laboratories. Identified outliers (up to seven) were excluded from the QC range establishment. New QC ranges were established for all but two reference strain-biocide combinations and comprise three to five dilution steps (Table 1). Due to the limited solubility of the biocides in the test range required for *P. aeruginosa* ATCC® 15442, QC ranges for chlorhexidine and polyhexanide could not be determined for this reference strain. Therefore, we recommend using *Escherichia coli* ATCC® 10536 as reference strain in parallel with *Pseudomonas* field isolates, when testing chlorhexidine and polyhexanide [1].

Table 1: Summary of the new quality control ranges (minimal inhibitory concentrations in %)

Biocide	*S. aureus* ATCC® 6538	*E. hirae* ATCC® 10541	*E. coli* ATCC® 10536	*P. aeruginosa* ATCC® 15442
Benzalkonium chloride	0.00003-0.00025	0.000125-0.0005	0.0005-0.002	0.002-0.008
Chlorhexidine	0.00003-0.00025	0.00003-0.00025	0.000015-0.00025	-
Polyhexanide	0.00006-0.001	0.000125-0.002	0.00006-0.001	-
Octenidine	0.00006-0.00025	0.00006-0.0005	0.00006-0.0005	0.000125-0.002

Conclusions: After developing harmonized BST methods [2], this study was the subsequent step. Together, these studies will contribute to the harmonisation and validation of the BST in the future.

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P79

Antibiotic resistance in Australian leafy greens pre-farm gate

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Fresh produce, including leafy greens are promoted as being a healthy source of vitamins and minerals, and are consumed daily worldwide. The increased consumption of fresh horticultural produce has been closely followed by an increase in foodborne disease outbreaks. Outbreaks caused by fresh horticultural produce has overtaken those caused by meat, dairy and seafood. This pathway can be a silent source of introduction and exposure for consumers, to antimicrobial residues, antimicrobial-resistant (AMR) bacteria and antimicrobial resistance genes (ARGs) of clinical importance. Exposure to AMR bacteria through the gastrointestinal tract may lead to drug resistant infections or the potential for the sharing of ARGs with other gut microflora eventually decreasing the effect of medicine and making infections harder to treat. So, the question is: are our leafy greens making us stronger and healthier or strengthening bacteria against antimicrobial treatment?

The major factors that contribute to the emergence and spread of AMR in leafy greens involve the use of antibiotics in plant production and livestock farming, as well as use of contaminated irrigation water, manure or soil. Moreover, the microbiomes of plants and soil that are involved in nutrient cycling, plant growth, and disease resistance, might also be acting as hotspots for the development and spread of AMR. Understanding the dynamics of microbial communities and their AMR profiles in horticultural systems is necessary to make informed strategies for reducing the risk of AMR. Knowledge of the presence and prevalence of AMR in the Australian horticulture sector is limited. So, the major aim of this project is to characterise the diversity of AMR genes and their distribution in commensal, plant pathogenic and food safety bacteria in the Victorian horticultural sector during pre-farm gate production and processing.

Metagenomic sequencing will be used to study the microbiome of leafy greens and associated antibiotic resistance genes (ARGs). The genes and mobile genetic elements will be identified, in both microbiome and from select bacterial isolates, to determine ARG prevalence in leafy greens. The AMR susceptibility of the select bacterial isolates will be confirmed to determine whether the ARGs are expressed. This PhD project will focus on:

1. The development of workflows to extract DNA from leafy greens for metagenomic sequencing
2. The development of bioinformatic pipelines to characterise the microbiome and associated ARGs
3. Studying the genetic links of ARGs and mobile genetic elements in select bacterial isolates to determine the possibility of horizontal gene transfer of ARGs in horticulture sector prefarm gate.

We are hopeful that this genotypic approach supported by phenotypic testing can provide important insights into the prevalence and diversity of ARGs in Australian horticulture pre-farm gate and help to mitigate the risks of AMR from farm-to-fork.

We are hopeful that this genotypic approach supported by phenotypic testing can provide important insights into the prevalence and diversity of ARGs in Australian horticulture pre-farm gate and help to mitigate the risks of AMR from farm-to-fork.

P80

Pyocin S2 Conjugates for the Treatment of PyoS2-Resistant MDR Clinical Isolates of *Pseudomonas aeruginosa.*

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The opportunistic human pathogen *Pseudomonas aeruginosa* is a Gram-negative bacterium that can be isolated from environmental sources, but is of great concern when found in a clinical context.[1] Widespread multidrug resistance (MDR) mechanisms against common antibiotics, reserve group antibiotics, and environmental toxins make *P. aeruginosa* difficult to treat and prompted the World Health Organization (WHO) in 2017 to list *P. aeruginosa* with priority 1 ("critical") on a list of pathogens, for which new antibiotics or promising alternatives are urgently needed.[2] An often mentioned potential alternative are so-called bacteriocins; however, bacteriocins of Gram-negative bacteria are lacking proof of applicability so far.[3] Bacteriocins are toxic multi-domain proteins and are found in most bacterial Gram-negative and Gram-positive species. Other than most small molecule antibiotics, bacteriocins are narrow band antibiotics, mostly acting on closely related strains of the same genus and are evolutionary relevant for the fight for ecological niches. Selectivity and host specificity rely on a conserved uptake mechanism, which involves the outer membrane protein machinery of the bacterial target cell. To date, several bacteriocins have been found in *P. aeruginosa* □ called pyocins □ that kill other strains of *P. aeruginosa*. The producing strain itself is protected from the pore-forming or nuclease killing activity of pyocins by co-expression of immunity proteins that confer resistance to this particular strain.[4] Here we present preliminary proof that resistance against the DNase pyocin S2 (PyoS2) can be overcome by partial blockage of the interaction site of the PyoS2 cytotoxic domain and its cognate immunity protein ImS2. *In vitro* labeled toxin with a non-toxic fluorophore label was shown to kill the PyoS2-producing *P. aeruginosa* PAO1 and several PyoS2-resistant MDR clinical isolates of *P. aeruginosa*. We believe that our findings might contribute to bringing bacteriocins of Gram-negative strains closer to application as potential substitutes for antibiotics or as extension of our common antibiotics portfolio for treatment of clinically relevant MDR strains in the future.

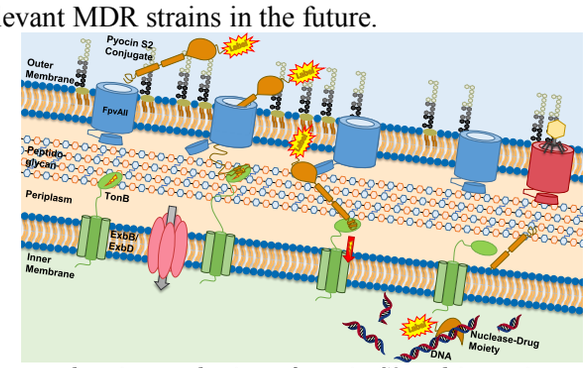

Fig. 1: *Schematic depiction of the translocation mechanism of pyocin S2 and its conjugates across the bacterial membrane of Pseudomonas aeruginosa. The outer membrane porin FpvAII serves as primary receptor and is hijacked by PyoS2 derivatives, the inner membrane TonB machinery energizes the import into the periplasm. Protease-assisted cleavage of pyoS2 leads to translocation into the cytoplasm, where the C-terminal cytotoxic domain of PyoS2 (DNase activity) causes cell death by degradation of genomic information.*

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P81

Impact of minocycline, an "old" antibiotic, on the digestive flora in pigs. A study model for humans.

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Introduction: The increase of multidrug-resistant (MDR) pathogens has renewed interest in old antibiotics. The combination of polymyxin B and minocycline for intravenous antibiotic therapy in human is promising. The drawback is the elimination of minocycline in the intestines, with potential impact on the digestive microbiota. Our objectives were to 1) determine the pharmacokinetic parameters of minocycline in pigs to mimic the expected exposure in humans and 2) assess the impact of minocycline administration on the intestinal microbiota.

Materials and Methods: Pigs received saline (Control group) or minocycline by intravenous route at a low dose (LD group, 8 mg/Kg/d for 4 days) or at high dose (HD group, 16 mg/Kg/d for 4 days). For the determination of pharmacokinetic parameters in the pharmacokinetic study (n=8 per group), minocycline concentrations in plasma were determined by U-HPLC with UV detection. For fecal microbiote evaluation, antibiotic resistance was evaluated by cultural methods and quantitative real-time PCR. The impact on microbial diversity was evaluated by sequencing the V3-V4 region of the 16S rRNA gene.

Results: Pharmacokinetic data indicated that minocycline was eliminated with a clearance 3 to 7 times higher in pigs compared to humans. Thus, in order to obtain exposure of pigs similar to that of humans, 8 to 16 mg/Kg/d were administered to pigs. High dose minocycline (16 mg/Kg/d) increased the proportion of minocycline-resistant *Enterobacterales* (-2.5Log to -0.5Log) after 4 days of treatment while the total (~10^5 UFC/g faeces) and minocycline-resistant *Enterobacterales* (10^2-10^3 UFC/g faeces) were unchanged. Quantification of the 95 genes coding for antibiotic resistance (minocycline, tetracycline, other antibiotic families) or genes linked to genetic mobile elements (transposon, insertion sequence, integron) showed the increase of the relative abundance of the *tet(G)* gene. No impact was detected on other genes. The majority of 16S rRNA sequences belonged to *Firmicutes* and *Bacteroides*. The beta-diversity index and nonmetric multidimensional scaling demonstrated changes in microbial membership after 4 days of minocycline treatment. Ninety-eight OTU were differentially abundant. However, bacterial phylotypes did not shifted with minocycline treatment showing similar rate of *Firminutes* and *Bacteroides* in treated or control pigs.

Conclusion: High dose minocycline treatment seems to have a weak impact on the digestive microbiote (resistance and diversity).

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P82

GenoFig: a user-friendly application for vizualisation and comparison of genomic regions.

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Most concerns about antibiotic resistance are linked to the extremely efficient dissemination of mobile genetic elements carrying resistance genes, such as plasmids and conjugative transposons. Understanding the evolution of these elements usually requires visual comparison of replicons hosted by various bacterial strains or species. Here we present GenoFig, a graphical application for the generation of comparative genomics figures, intended to be as easy to use as possible for biologists and flexible enough to adapt to a variety of needs. GenoFig allows the personalized representation of annotations extracted from GenBank files in a consistant way across sequences, using regular exressions. It also provides several unique options to optimize the display of homologous regions between sequences, as well as other more classical features such as GC percent or GC-skew representations. In summary, GenoFig is a simple, free, and highly configurable tool to explore the evolution of specific genomic regions and to produce publication-ready figures.

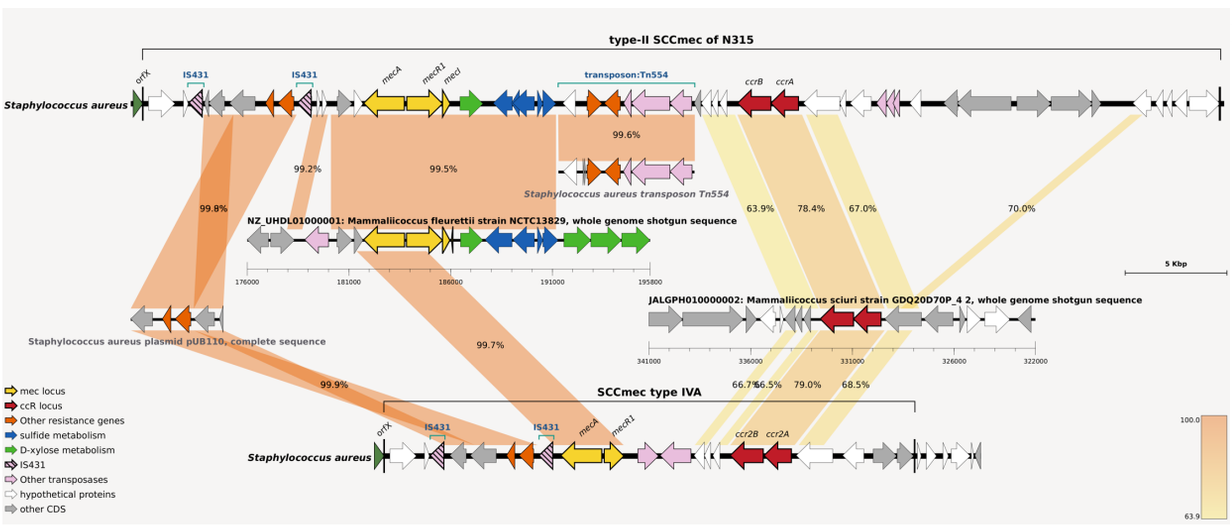

Fig. 1. Example of figure produced with GenoFig

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Quantification and molecular characterization of vancomycin resistant Enterococcus faecium (VRE) in hospital wastewater before, during and after a hospital outbreak

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Background and Objectives. With the COVID-19 pandemic, sewage-based surveillance as a monitoring tool of rare micro-organisms in the human population has gained more interest globally. Sewage-based surveillance is typically performed at the municipal wastewater treatment plant level to cover large populations. The current study aimed to investigate sewage-based surveillance at an institutional level as a tool to support the detection and management of hospital outbreaks and infection prevention and control, focusing on vancomycin-resistant *E. faecium* (VRE).
Methods. Wastewater was sampled from a large Dutch teaching hospital, between March 2022 and April 2023, using an automated sampler at a baseline frequency of once per month. Between 14 November 2022 and 6 March 2023 the hospital was confronted with a VRE outbreak involving 18 patients at three wards. Because of this, the sampling frequency was increased to once weekly as of January 2023. Wastewater samples were membrane filtered, followed by enrichment in vancomycin-supplemented broth and subsequent plating on selective agar. Initially, a semi-quantitative detection method was used. As of September 2022, the numbers of VRE were determined using a most probable number method. Suspected VRE colonies were confirmed using MALDI-TOF, E-test and vancomycin-PCR. From the start of the outbreak all hospital patients at affected wards were screened weekly for the presence of VRE, and a contact investigation was done after finding a new case. Whole genomes of VRE isolates from patients and wastewater were sequenced using Illumina Miseq and compared using cgMLST analysis using a cluster distance of 20 nucleotides. VRE concentrations and cgMLST variants in wastewater were analysed relative to the presence of VRE-positive patients before, during and after the outbreak.
Results. VRE was detected in all 20 wastewater samples. Before the outbreak, four different unrelated sequence variants were identified at different time-points, among which the outbreak variant (3/10 isolates). From two months before the outbreak till the end of the outbreak, VRE concentrations ranged between 10^5 - 10^6 MPN per litre and only the outbreak variant was detected (12/12 isolates). After that, VRE concentrations declined quickly to 10^3 MPN per litre and variant variability was observed again, consisting of the outbreak variant (1/3 isolates) and one of the pre-outbreak variants. These results are preliminary, as not all sequence data were available at the time of writing.
Conclusion. High VRE concentrations in wastewater preceded the identification of the hospital outbreak in patients, and concentrations subsequently followed the outbreak dynamics. During the outbreak, VRE sequence types of the isolates shifted from multiple unrelated types to solely the outbreak-related type. Although further investigation is required, these aspects suggest that wastewater surveillance may be a valuable tool to support the management of hospital outbreaks.

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ABRomics: An integrated multi-omics platform for antibiotic resistance research and public health

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Antibiotic resistance (ABR) is a major public health issue prioritized for mitigation by international institutions. Multidrug resistant bacteria (MDRB) and Antibiotic Resistance Genes (ARGs) carried by mobile genetic elements spread between the human, animal, and environmental sectors. Whole Genome Sequencing (WGS) is used for molecular typing purposes at the highest resolution. It provides identification of ARGs and their genetic supports as well as mutations leading to a decrease in antibiotic susceptibility. Epidemiological and WGS data are used for tracking MDRB in hospital outbreaks but also across the animal and environmental sectors. Sharing and interoperability of high-quality data (sequence and metadata) are key requirements for addressing the spatio-temporal dissemination of MDRB. To this aim, the French Priority Plan on ABR has funded the development of an online, open platform dedicated to antibiotic resistance.

We are establishing a repository of structured, interoperable, standardized, and well-annotated multi-omics data with tailored mathematical and bioinformatics tools to answer generic and specific research questions related to ABR. The ABRomics platform includes standardized pipelines to run ABR analyses of WGS from pathogenic strains supported with integrated databases (ARG, sequence types [ST], virulence factors [VF]). Uploading data, launching pipelines, viewing and cross-referencing enriched results will be achieved through easy-to-use web interfaces. ABRomics ß-version integrating the ABR detection genomic pipeline and other markers such as ST, and VF will be available to the consortium in summer 2023 and to the whole microbial research community by the end of 2023. Core-genome multi-locus sequence typing, relationships between strains and metagenomics pipelines will next be made available.

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Session 5 - Novel approaches, methods and tools dedicated to antimicrobial resistance (detection, evolution, diagnostics, surveillance)

P85

Phenotypic and WGS-based characterization of antimicrobial resistance of *Trueperella pyogenes* clinical isolates from humans and animals in Switzerland

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Trueperella pyogenes, a commensal Gram-positive facultative-anaerobic rod of the skin and mucous membranes of animals can cause suppurative infections in animals, and rarely humans (1). These infections are commonly treated with antibiotics, posing the risk of selecting resistant bacteria through the acquisition of mobile genetic elements containing antimicrobial resistance genes (2). The objectives of the present study were to determine i) the phenotypic and genotypic antimicrobial resistance profiles of *T. pyogenes* in Switzerland, and ii) the genetic relatedness between strains of different origins.

Thirty-five *T. pyogenes* isolates from animals and 8 from humans were obtained from a veterinary and a human diagnostic facility in Switzerland. The strains were cultivated on TSA-S (Becton, Dickson) under 5% CO_2 atmosphere at 37°C for 24 hours. Identification was confirmed by MALDI-TOF mass spectrometry (Bruker). Minimal inhibitory concentrations (MICs) of 19 antibiotics and 3 antiseptics were determined by broth microdilution method following CLSI recommendations. The MICs were interpreted using breakpoints derived from previously published data. Genomic DNA was extracted using Masterpure™ purification kit (Lucigen) and sequenced at the NGS Platform, University of Bern, Switzerland, using PacBio HiFi technology. Reads were demultiplexed and *de novo* assembled using Flye v.2.9.1. The 43 complete genomes were screened *in silico* for antimicrobial resistance genes using Resfinder v.4.1 (Center for Genomic Epidemiology) and CARD-RGI (McMaster University). Core genome-based MLST (cgMLST) analysis was performed for phylogenetic relatedness.

Seventeen isolates were susceptible to all tested antibiotics. Resistant test results to tetracycline (n=23), streptomycin (n=20), and sulfonamides (n=17) were common in both animal and human isolates, while resistance to erythromycin (n=4), clindamycin (n=3), trimethoprim (n=2), and chloramphenicol (n=2) was only sporadic and predominantly detected in animal isolates. The resistance genes *tet*(W), *aadA9*, and *sul1* were the most frequently detected, reflecting the phenotypic resistance profile. Phenotypic resistance to quaternary ammonium compounds was not observed despite the frequent detection of the resistance gene *qacEΔ1*. Two large clusters were identified by cgMLST, revealing that human isolates were more closely related to those from cattle compared to other animal species.

The presence of genetically related *T. pyogenes* strains in humans and animals suggests that exchange of mobile genetic elements may occur. Acquired resistances to several antimicrobials indicates that judicious use of antibiotics should be made based on antimicrobial susceptibility testing.

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Antimicrobial resistance trends of *Salmonella* spp. isolated from yellow-legged gulls (*Larus michahellis*) from Medes Islands (Catalonia, NE Spain)

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Salmonellosis is the second most commonly reported foodborne gastrointestinal infection in humans in the EU. Not only food animals are carriers of *Salmonella*, but also wildlife. These particularly include many gull species that scavenge on refuse and can act as *Salmonella* reservoirs, including their antimicrobial resistant (AMR) strains. Our former study already showed the role of yellow-legged gulls (*Larus michahellis*) in the epidemiology of *Salmonella* and their AMR in southern Europe. Here, we have monitored one colony of this gull with a higher anthropogenic pressure in NE Spain, the Medes Islands, to assess its occurrence and trend of AMR *Salmonella*. We have focused on the most relevant *Salmonella* serovars responsible of human salmonellosis in the EU, that is, Enteritidis, Typhimurium and its monophasic variant. Assessment of the AMR of isolates (n= 38) recovered in 2013, 2017, 2018, 2020, 2021 and 2022 has been performed by means of a broth microdilution method using EUSVEC plates. Overall *Salmonella* spp occurrence over the years peaked at 80% in 2020 and bottomed out at 38% and 33%, in 2013 and 2021, respectively. Serovar Enteritidis was the less frequent, being isolated only in 2013. On the contrary, ser Typhimurium was isolated in 2013, 2017 and 2022, while its monophasic variant was the most frequent, and was isolated in all years. Overall, a high prevalence of AMR was found, with 95% of tested isolates showing resistance to at least one antimicrobial. Also, the prevalence of multidrug resistance (MDR) was high (55%), with monophasic *S.* Typhimurium showing the highest prevalence (63%), while *S.* ser. Enteritidis did not show MDR. However, a decreasing trend of MDR in the monophasic variant was observed across years (from 100% in 2013 to 33% in 2022). The most prevalent MDR profile was Ampicillin-Tetracycline-Sulfamethoxazole. However, there were isolates showing resistance up to 5 antimicrobial classes, although with a very low frequency. The three *S.* ser. Enteritidis showed resistance to quinolones (n=2) or to sulfamethoxazole alone (n=1). The decreasing trend of AMR shown by the monophasic variant of *S.* Typhimurium might be a result of the important reduction of antimicrobial use in both veterinary and human medicine during last years in Spain (and the EU in general). These findings highlight the importance of gulls in the epidemiology of AMR and support the potential of yellow-legged gulls as sentinels for environmental AMR in the Mediterranean region and the need to tackle this public health concern from a One Health approach.

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Gulls in southern Spain as carriers and potential spreaders of *Salmonella* spp. and antibiotic-resistant strains

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Some gull species, particularly those with scavenging feeding habits, can act as reservoirs of infectious agents and play an important role in their dissemination and maintenance in the environment [2]. This includes zoonotic agents such as *Salmonella,* which in turn can show resistance to antimicrobials of veterinary or human relevance. To gain insight into the epidemiology of *Salmonella* spp. and their antimicrobial-resistant strains, isolates recovered during 2022-2023 from two different large gull species in southern Spain were analysed. A total of 369 birds were sampled in urban or nearby urban areas: *Larus fuscus* (n=134) and *Larus michahellis* (n=235). To assess the presence of antibiotic-resistant (AMR) strains, the isolates were characterized phenotypically using a broth microdilution method using EUSVEC3 plates. Overall, 4,87% (18/369) of the individuals were *Salmonella* carriers, with *L. fuscus* showing the highest prevalence (7,4%) followed by *L. michahellis* (3,4%). A high diversity of serovars was identified, with each gull species carrying different serovars: Bovismorbificans, Derby, Isangi, London, and Poona, in *L. fuscus*; Virchow, Brandenburg, Typhimurium monophasic, and Stanley in *L. michahellis*. Overall, 22,22% (4 out of 18) of *Salmonella* isolates were resistant to at least one antimicrobial agent and belonged to serovars Virchow and monophasic *S.* Typhimurium for *L. michahellis*, and ser. Bovismorbificans for the single resistant isolate from *L. fuscus*. The single multidrug resistant strain was recovered from *L. michahellis* and belonged to the serovar monophasic *S.* Typhimurium, with the AMR profile ampicillin-tetracycline-sulfamethoxazole. We have also commonly found this AMR profile in isolates from monophasic *S.* Typhimurium carried by *L. michahellis* from a densely populated colony in northeastern Spain. Besides, of the two gull species sampled, *L. michahellis* carried three out of the four AMR strains found. Despite the relatively low prevalence of *Salmonella* in both gull species, it is noteworthy the prevalence of AMR strains. These results highlight gulls as potential spreaders of AMR strains in the environment that may pose a risk for public health, especially in human populated areas, as also shown in other gull colonies in southern Europe [1]. It also supports the potential role of gulls as sentinels for AMR in the environment.

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Ambulatory decolonization of a cat carrying Panton-Valentine leukocidin (PVL)-producing *S. aureus*

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Introduction: Panton-Valentine leukocidin (PVL)-producing *Staphylococcus aureus* (PVL-SA) is a frequent cause of skin abscesses in humans. Besides antibiotics and surgical treatment, topical decolonization is a preventive measure for reducing the risk of PVL-SA recurrence and transmission. Here, we report on an affected family of four that was treated but continued to suffer from repeated PVL-SA positive skin infections even after three courses of appropriate decolonization and whose two cats were identified to be colonized by *S. aureus* (SA). Although dogs and cats can be potential carriers of PVL-SA and hence a source of reinfection for humans, validated protocols for decolonization of animals are not available. Thus, a protocol for outpatient decolonization using systemic antibiotic treatment of cats was developed.
Methods: For bacteriological examination, samples from both cats were obtained from the nose, oral cavity, rectum, inguinal and perianal region. SA isolates were tested for the presence of *lukF-lukS* by PCR and subjected to susceptibility testing (AST) according to CLSI. Comparative analysis of human and feline isolates was performed by whole genome sequencing. Results of susceptibility testing were used to develop a decolonization protocol based on systemic therapy with amoxicillin-clavulanic acid for 10 d and 20 d, respectively.
Results: Methicillin-susceptible SA was isolated from the oral cavity and nose of both cats. While one cat was a carrier of PVL-SA, a PVL-negative SA strain was isolated from the second cat. Comparative whole genome analysis revealed close clonal relationships of both the PVL-SA assigned to sequence type (ST 8) and a further SA (ST45) isolated from human and feline samples. In the course of oral decolonization therapy, a significant reduction of SA was accomplished. Although the samples of the SA-carrying cat were negative after 10 days, the PVL-SA-positive cat required a second course of antibiotics (amoxicillin for 18 days). Control examinations of the cats after 3 and 7 weeks were negative for SA.
Discussion: In the case presented, successful decolonization of cats colonized with PVL-positive or -negative *S. aureus* was achieved by a combination of systemic antibiotic therapy and hygiene measures. The close relationship of human and feline isolates suggests transmission between humans and animals in the household and underscores the importance of potentially colonized pets for the success of decolonization measures.

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Starved of ACTION: A Critical Look at the Antimicrobial Resistance Action Plans of African Countries

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The urgent need for Africa, as a continent, to start galvanizing resources and strengthening its capacity to win the fight against the looming threat of antimicrobial resistance (AMR) was once again brought to the limelight by the recent study on the "Global Burden of Bacterial Antimicrobial Resistance" (The Lancet2022, 399, 629). According to the study, western sub-Saharan Africa is a super-region for AMR, with overall mortality rates due to and associated with AMR standing at 27.3 and 114.8 per 100 000 people, respectively. Putting this in perspective, if the leaders of this region do not take effective action, Africa will indeed be one of the two worst-affected regions, along with Asia, with the possibility of over 4.1 million people dying annually from AMR by 2050. The development of a National Action Plan, as directed by the World Health Organization, provides an important framework for addressing the complex and multi-faceted nature of the rise and spread of drug-resistant bacteria. This paper reviews the AMR National Action Plans of African countries and calls on the leaders of this region to move from paper to action and to look beyond just the reduction of antibiotic consumption and, as a more comprehensive response to the threat of AMR, focus also on expanding access to safe drinking water, bettering sanitary conditions, maintaining effective leadership at all levels of governance, increasing government spending on healthcare services, and strengthening regulatory oversight.

Reference

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Multidirectional dynamic model for the spread of Extended-Spectrum-β-lactamase-Producing *E. coli* in the Netherlands

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This work aimed to design a multidirectional dynamic risk model with which the contribution of different sources to the extended-spectrum β-lactamase *Escherichia coli* (ESBL-EC) prevalence in humans can be assessed. A discrete-time model was built to describe within and between population spread of ESBL-EC in humans (open community), farmers, broiler flocks and veal herds. The slaughterhouse and consumer phase (food preparation) were included in the model to account for ESBL-EC transmission via food consumption. Recreational surface water was added as a source of ESBL-EC for humans when swimming in surface water. The model was parameterized for the Netherlands. Data availability was limited and a sensitivity analysis was conducted to explore the effect of parameter uncertainty on model results. The average ESBL-EC prevalence in the open community, broiler farmers and veal farmers after simulating transmission for 200 weeks was 2.1%, 20.1% and 47.3%, respectively. The most relevant sources for ESBL-EC colonization of the open community were the open community itself, contact of the open community with veal farmers, and consumption of vegetables cross-contaminated by chicken meat with 59.4%, 18.5%, and 12.5%, respectively (Fig. 1). The uncertain parameters that most affected model results were the colonization and decolonization rates for humans.

Fig. 1. Relative source attribution to the different populations included in the multidirectional model for ESBL-EC spread in the Netherlands. NA: source not considered for this population.

Results of the model contribute to a better understanding of the relative importance of sources contributing to ESBL-EC prevalence in humans. The model, however, also accounts for humans' contribution to the ESBL-EC prevalence in these sources, resulting in a One Health approach for risk assessment of antimicrobial resistance transmission. The model results can inform public health policymakers to mitigate antimicrobial resistance risk.

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Focus on data and modelling practice – a systematic review of AMR transmission model.

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Mathematical transmission models are a valuable tool to guide intervention strategies in public health. Literature reports a number of systematic reviews on antimicrobial resistance (AMR) transmission models with different aims. Here we systematically search, and review publications modelling the transmission of antimicrobial-resistant bacterial pathogens in the human population and evaluate models by the TRACE framework and thereby identify gaps in the modelling of AMR.
A total of 168 studies were identified and included in the review. The most targeted WHO regions were the European region and the region of the Americas. *Mycobacterium tuberculosis* (n = 40) and *Staphylococcus aureus* (n = 38) were the most modelled pathogens, with a focus on multi-drug and methicillin resistance, respectively. A large fraction of the studies modelled the effects of interventions, hereof 1/3 with more than one intervention. Half of the studies modelled symptomatic infections, here most of them specified a specific infection site. Most of the included studies were population-based compartment models (n = 110). Models describing validation and sensitivity analysis (n = 39) were evaluated by applying the TRACE framework. The evaluation showed a general lack of validation of models both internally and with external data. Our review highlights the importance of describing good modelling practices and the need for more data to compare with the model outputs.

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PROMISE, a One Health meta-network to fight antibiotic resistance in France

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Introduction and objectives:
Antimicrobial resistance (AMR) is a major health issue affecting humans, animals and ecosystems. To fight effectively against AMR, we need to adopt a One Health approach taking into account the interconnection between these 3 sectors. However, the operationalization of this concept often remains difficult due to the differences between these 3 worlds.
Launched in 2021 and supported by the French priority research program on AMR, the PROMISE project (https://amr-promise.fr/fr/) brings together the main actors involved in the fight against AMR in France (25 professional networks and more than 40 research units, from the 3 sectors) to build a large One Health community on AMR. PROMISE aims to build interactions between actors usually working in silo and allows its members to coordinate their actions.
Methods:
PROMISE's action is based on 7 transdisciplinary pillars:

- Knowledge and skills sharing
- Data sharing
- AMR surveillance
- Clinical research
- Communication and awareness
- Training
- Structuring of emerging networks in areas lacking coordination

Results, conclusion:
The main results of PROMISE include:

- A cross-sectoral analysis of the correlations between consumption and resistance in France;
- The creation of a common One Health data warehouse gathering surveillance data from the 3 sectors and paving the way for a more integrated AMR surveillance.
- The launch of large-scale clinical studies involving the 4 main clinical research networks of intensive care units and infectious diseases in France;
- The organization of events allowing the community to come together;
- The launch of a diploma course allowing professionals from the 3 sectors to learn about AMR and One Health
- The coordination of a new network dedicated to the operationalization of a AMR monitoring system in the environment
- The structuration of a network dedicated to preclinical research in antibiotic therapy

To conclude, PROMISE is a unique opportunity to structure the AMR community and make France a best practice region in Europe in terms of One Health coordination.

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Coalition of Non-Profit Organizations Pushing for Policy Change in the United States' Industrial Agriculture Sector

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The Keep Antibiotics Working (KAW) Coalition fights against the spread of antibiotic-resistant superbugs. Formed in 2001, KAW is a coalition of nineteen advocacy groups that joined to ensure that untreatable superbugs resulting from the overuse of antibiotics on farms do not reverse the medical advances of the past century. KAW urges that it is more critical than ever for the United States Food and Drug Administration (FDA) and the United States Department of Health and Human Services (HHS) to confront the slow-moving resistance pandemic, which often spreads via animals and is exacerbated by livestock husbandry practices. We need information about how and why antibiotics are used across all settings, in order to fully understand and respond to ongoing antibiotic overuse – one of the largest, if not the largest driver of the spread of resistance. Curbing medically important antibiotic use in food-producing animals is especially important; around two-thirds of the nation's medically important drugs are sold for use on animals, not people. [1] The FDA must begin to robustly monitor antibiotic use, but also prioritize actions to effectively curb the overuse of these precious medicines. KAW organizations advocate for government action to control the overuse of on-farm antibiotics. Specifically, we recommend that the FDA and HHS commit to the following actions to protect public health.

1. Establish a national stewardship goal to curb medically important antibiotic sales for use in food-producing animals by at least 50% by the end of 2025, relative to 2010 levels.
2. End administration of antibiotics to entire herds where there are not sick animals. The U.S. finished phasing out antibiotics for growth promotion in 2017, but use of the same drugs in healthy animals for disease prevention continues to be legal. In order to promote stewardship preventive use should be disallowed, except in the case of surgery. This will slow the emergence of antibiotic resistance and encourage higher welfare standards in farming operations.
3. Develop comprehensive, national systems to monitor and report pertaining to farm-level antibiotic use and resistance, using a One Health approach. The continued lack of national systems to track antibiotic use at the farm level leaves a critical gap in antibiotic stewardship efforts and national pandemic preparedness.
4. KAW expects farms to prevent disease by providing appropriate diets, delaying weaning, improving hygiene, reducing crowding, and using vaccines appropriately-so that antibiotic use on farm is rare not routine.

References

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Contribution to the control of antimicrobial resistance in human and animal health in Togo: a One Health approach

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Antibiotic resistance among bacteria in West Africa is a major concern. In Togo, no studies have precisely characterized isolated multidrug-resistant bacteria or compared their genomes to identify transmission pathways. It is within this context that our study is conducted.
During this study, we assess the prevalence of carriage of extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL) and carbapenemase-producing Enterobacteriaceae (CPE) in farmers and their animals, as well as the possibility of inter-species transmission of strains. To achieve this, samples are collected from farmers and their animals to isolate and compare phenotypically and genotypically the resistant bacterial strains. Additionally, an epidemiological and socio-economic survey conducted in parallel allows us to potentially highlight risk factors associated with inter-species transmission and evaluate the acceptability of a rational antibiotic use plan in livestock farming.
The main objective of our study is to estimate the prevalence of ESBL and CPE carriage in farmers and their animals living in the maritime region of Togo. The secondary objectives are to identify risk factors associated with ESBL and CPE carriage in animals, characterize the species producing ESBL and CPE, as well as the enzymes responsible for resistance, and assess the acceptability of a plan to reduce antibiotic use in livestock farming.
The methods of our study involve administering a questionnaire to farmers to list the risk factors for the emergence of ESBL/CPE in livestock farming and assess the farmer's level of investment in antimicrobial use and their willingness to consider alternative methods for controlling animal diseases through a semi-structured interview. All farmers who refuse to participate in the study are excluded. Fecal samples are collected from 160 animal productions, as well as 80 stool samples from farmers, either by rectal swabbing or using a stool potty. The detection of ESBL and CPE is performed on MacConkey agar supplemented with cefotaxime and MacConkey agar supplemented with ertapenem, respectively. Bacterial colonies are identified using mini-galleries or a complete gallery (API 20E). Antibiotic susceptibility testing is also conducted. The identification of resistance mechanisms is performed using either phenotypic methods (ESBL) or genotypic methods (Genexpert, Cepheid for CPE). Genomic analyses are also carried out through whole-genome sequencing (Miniseq, Illumina).
Regarding the results, the survey is currently still ongoing. However, ESBL and CPE have been isolated in 91.9% and 8.1% of the first 37 investigated farms, respectively. Among the multidrug-resistant bacteria (MDR) present in the farms, a significant proportion of *E. coli* (54.1%) and *Raoutella ornitholytica* (13.5%) have been observed.

Notes

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