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# Abstract booklet

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ARAE

中國農業大學动物医学院
College of Veterinary Medicine, China Agricultural University

Contents

# Keynote Lecture

## KL-001

## Clinical Breakpoints for Veterinary Antibiotics – Essential to Antibiotic Stewardship

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**Background and objectives:** Accurate and updated clinical breakpoints for antimicrobial susceptibility testing (AST) are needed to predict clinical response to veterinary antimicrobial agents. They are also essential for antimicrobial stewardship.1

**Methods:** In the past 30 years, the Clinical and Laboratory Standards Institute (CLSI), Veterinary Antimicrobial Susceptibility Testing subcommittee (VAST) (www.CLSI.org) has developed veterinary specific clinical breakpoints for bacteria isolated from animals. In the last edition of VET01[2] there are over 280 approved clinical breakpoints approved through a consensus-driven process by a committee of experts. In addition to providing new breakpoints for antimicrobial agents used in veterinary medicine, we updated older breakpoints based on new information on pharmacokinetics-pharmacokinetics (PK-PD) and microbiologic data or shifted from using the human breakpoint to a veterinary-specific breakpoint. These new breakpoints will improve clinical therapy with more accurate susceptibility testing and decrease the risk of emergence of resistance. Our methods are described in CLSI documents and published in other papers, most recently for the fluoroquinolone antimicrobials[3-5].

**Results:** Through an examination of the published literature, and examination of Freedom of Information (FOI) summaries available from the U.S. FDA, our CLSI subcommittee collected pharmacokinetic data for antimicrobial agents used in veterinary medicine. The PK-PD and Monte Carlo simulations were used to develop new breakpoints for these antimicrobial agents[2]. (Table 1)

One of the changes was for breakpoints to test fluoroquinolone antimicrobials for isolates from dogs 5. New breakpoints for these agents to test feline isolates are pending. These changes from the prior breakpoints reflect more current understanding of the PK-PD properties of fluoroquinolones, more pharmacokinetic data, and a large database of microbiology data not available previously.

The accompanying table shows the new Susceptible Dose-Dependent (SDD) category, which requires a higher dose. (Similar to the "I" category used by EUCAST, to mean *increased exposure*.) With these breakpoints, isolates that previously may have tested S, will now be interpreted as R.

A new breakpoint was also included in the 7th edition of the VET01(S)[2] standard for chloramphenicol. This was developed to reflect current understanding of PK-PD principles for chloramphenicol, and new data on MIC distributions. This replaces the old human breakpoints of S, ≤ 8 µg/mL; I, 16 µg/mL; and R, ≥32 µg/mL. At this new breakpoint, very few isolates from dogs will test susceptible. Other breakpoints under revision, or yet to be approved by the CLSI committee, are the breakpoints for ampicillin (IV).

**Conclusions:** The on-going efforts of the CLSI-VAST subcommittee to develop new clinical breakpoints for bacteria from animals will improve the testing standards used in clinical laboratories. The revised and updated testing standards aligns with the CLSI mission of improving clinical therapy and supporting antimicrobial stewardship.

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## Figure

**Table 1: New Interpretive Categories for Antimicrobial Agents in Dogs and Cats**

| | Breakpoints (µg/mL) | | | |
|---|---|---|---|---|
| | S | I | SDD | R |
| **Dogs** | | | | |
| Enrofloxacin (Old) | ≤ 0.5 | 1–2 | | ≥ 4 |
| Enrofloxacin (New)[a, b] | ≤ 0.06 | | 0.12–0.25 | ≥ 0.5 |
| Levofloxacin | ≤ 0.5 | 1 | | ≥ 2 |
| Marbofloxacin (Old) | ≤ 1 | 2 | | ≥ 4 |
| Marbofloxacin (New)[a, b] | ≤ 0.12 | | 0.25 | ≥ 0.5 |
| Pradofloxacin | ≤ 0.25 | 0.5–1 | | ≥ 2 |
| Chloramphenicol (Old) | ≤ 8 | 16 | | ≥ 32 |
| Chloramphenicol (New)[c] | ≤ 2 | 4 | | ≥ 8 |
| Ampicillin (human) | ≤ 8 | 16 | | ≥ 32 |
| Ampicillin (proposed)[d] | ≤ 0.25 | 0.5 | | ≥ 1 |
| Ampicillin (proposed)[e] | ≤ 2 | | 4 | ≥ 8 |
| **Cats** | | | | |
| Enrofloxacin (Old) | ≤ 0.5 | 1-2 | | ≥ 4 |
| Enrofloxacin (New)[a] | ≤ 0.12 | 0.25 | | ≥ 0.5 |
| Marbofloxacin (Old) | ≤ 1 | 2 | | ≥ 4 |
| Marbofloxacin (New)[a, b] | ≤ 0.25 | - | 0.5 | ≥ 1 |
| Pradofloxacin | ≤ 0.25 | 0.5–1 | | ≥ 2 |

a. Susceptible fluoroquinolone dose: 5 mg/kg once daily, enrofloxacin; 2.75 mg/kg once daily, marbofloxacin.
b. SDD fluoroquinolone dose: 10-20 mg/kg once daily, enrofloxacin; 5.5 mg/kg once daily marbofloxacin.
c. Chloramphenicol dose: 50 mg/kg, oral, q8h.
d. Ampicillin dose: 20 mg/kg IV, q8h (or ampicillin-sulbactam)
e. Ampicillin SDD dose: 100 mg/kg IV q8h.

**KL-002**

## Biocides as drivers for the selection and evolution of antimicrobial resistance

Frank Schreiber[1]

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Antimicrobial resistance (AMR) is a global health problem with the environment being an important compartment for the evolution, selection and transmission of AMR. These processes are impacted by pollution with antibiotics. However, antimicrobial biocides used as disinfectants and material preservatives are major pollutants exceeding the antibiotic market in terms of chemical diversity and mass. The aim of our work is to understand the mechanisms and risks of biocides for resistance and antibiotic cross-resistance evolution in bacteria to optimize their application and safeguard their efficacy. We use adaptive laboratory evolution experiments, phenotypic characterization, single-cell analysis, whole genome sequencing, and competition experiments to investigate AMR evolution and selection of the model organism *E. coli* in the presence of biocides. Our work shows that biocides have the potential to affect evolutionary processes towards AMR by increasing the rates of de-novo mutation and conjugation. Importantly, widely used compounds such as chlorhexidine and quaternary ammonium compounds (QACs) affect rates of mutation and conjugation at environmentally relevant concentrations. Furthermore, we show that single-cell phenotypic heterogeneity regarding tolerance (persistence) determines survival against specific biocides including QACs and isopropanol. Mechanistic investigations reveal that known antibiotic persister mechanisms contribute to persister formation to biocides. The evolution of high-level tolerance to different biocides is linked to the initial persister level and the evolution of specific genetically encoded mechanisms related to properties of the cell envelope. Biocide-tolerant strains have a selective advantage in the presence of environmentally-relevant concentrations of antibiotics, which could lead to the stabilization of biocide tolerance in environments where biocides and antibiotics co-occur (e.g. wastewater, animal stables). Taken together, our work shows the importance of assessing the contribution of biocides on evolution and selection of AMR in the environment.

**KL-003**

## Control technologies to prevent spread of environmental antibiotic resistance in the production and use of veterinary antibiotics

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**Background and objectives:** Fermentation production and use of veterinary antibiotics were the hotspots of antimicrobial resistance (AMR) dissemination in the environment. Here, we aimed to uncover the contamination of antibiotics and development of AMR during the treatment of antibiotic production wastewater and livestock wastewater, and develop novel technologies to block the release of these pollutants to the environment.

**Methods:** Full-scale investigation of AMR characteristics in treatment facilities of antibiotic production wastewater and livestock wastewater was conducted, and selective removal techniques of antibiotics and clinically relevant antibiotic resistance genes (ARGs) were developed.

**Results:** The widely used biological systems, usually a combination of anaerobic digestion and activated sludge process, are vulnerable to the presence of extremely high concentrations of

antibiotics in antibiotic production wastewater. Heavy multidrug resistance would be developed during wastewater treatment due to horizontal gene transfer of ARGs among bacterial community mainly through the enrichment of plasmids harboring multidrug resistance regions. Based on the above findings, pre-treatment of production wastewater to remove antimicrobials is the best way to control the development of AMR during the biological wastewater treatment. Enlightening by the easy-to-hydrolyze property of most of antibiotics, we have developed a novel pretreatment technology based on enhanced hydrolysis by using homogeneous or heterogeneous solid acid/base catalysts for targeted elimination of antibiotic potencies from wastewater. Then this technology has been successfully applied to the treatment of oxytetracycline manufacturing wastewater in two sites in Hebei Province (800 m3/d and 1,000 m3/d, respectively). The abundance of ARGs in the biological treatment units could be reduced by more than 83%, and the challenge on biological inhibition was also solved. In addition, hydrothermal treatment based on enhanced hydrolysis was also applied in full-scale plants in China for recycling waste erythromycin and cephalosporin fermentation residues. Livestock wastewater was another major discharge source of ARGs in the environment. About 300 *bla*CTX-M-carrying *E. coli* strains and *optrA*-carrying *Enterococcus* strains were isolated from mesophilic anaerobic digestion effluents treating manure wastewater. Mobile *tet*(X4) was also found as the dominant *tet*(X) variant, and persisted within anaerobic digestion treatments. Hyperthermophilic-mesophilic two-stage anaerobic digestion could reduce the abundance of *bla*CTX-M and *optrA*, and inactivate the fecal bacteria effectively, indicating effective management of operating temperature in anaerobic digestion should be implemented to prevent the discharge of the clinically relevant ARGs from the animal farms.

**Conclusions:** Antibiotic production wastewater pretreatment technology based on enhanced hydrolysis and livestock wastewater treatment technology based on hyperthermophilic-mesophilic two-stage anaerobic digestion were developed to prevent the spread of environmental antibiotic resistance.

This study was supported by the National Natural Science Foundation of China (NSFC) projects (32141002).

**KL-004**

# Molecular epidemiology of LA-MRSA: tracking transmission between animals and humans

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Antimicrobial agents are important for the treatment of bacterial infections not only in humans, but also in animals. Many antimicrobial classes used in animals are the same as those used in human medicine. Following extensive use in humans and animals, acquired resistance among pathogens and commensal bacteria has emerged. Bacteria harboring resistance to antimicrobials can be found in animals, food products and the environment. Humans can be exposed to antimicrobial-resistant bacteria originating from animals through a wide range of sources and transmission pathways, e.g through direct contact with animals, through consumption of contaminated food products or through exposure through the environment. Therefore it is important to address antimicrobial resistance in a One Health context. One example of resistant bacteria that can be transmitted between animals and humans are *livestock-associated methicillin-resistant Staphylococcus aureus (LA-MRSA).*

The Netherlands is a country with a low endemic level of MRSA in humans due to the restricted use of antibiotics and implementation of a so-called Search and Destroy policy. This policy includes active screening of high-risk groups upon hospital admission, preventive isolation, and treatment of MRSA carriers. For the Dutch national MRSA surveillance in humans, medical microbiology laboratories send MRSA isolates from carriers and from infected persons to the National Institute for Public Health and the Environment. During the last decades, livestock has emerged as a major source of MRSA, colonizing and infecting humans worldwide. The epidemiology of LA-MRSA differs in different

geographic regions. In Europe, MRSA clonal complex (CC)398 is the predominant CC found in livestock. The most important risk factor for carriage and infections with CC398 in humans is (professional) contact with livestock. Recently, however, the number of persons colonized or infected with MRSA CC398 in the Netherlands who did not have direct contact with livestock, seems to be increasing. In addition, Panton–Valentine leucocidin (PVL) positive MRSA CC398 are increasingly found in the Dutch MRSA human surveillance with patients predominantly living in regions with few livestock farms. PVL is a cytotoxin associated with skin- and soft tissue infections. As the epidemiology of MRSA CC398 seems to be changing, there is a need for increased molecular surveillance in humans as well as animals. In 2018, a MRSA surveillance study on livestock farms was set up in the Netherlands. This study investigated the prevalence of MRSA on broiler-, pig-, veal calf-, dairy cattle- and sheep farms, persons living and/or working on these farms and on retail meat. To assess possible transfer of resistant strains/resistance genes, animal-related CC398 MRSA were characterized in detail and compared those from the Dutch national MRSA surveillance in humans.

In this presentation the results of the Dutch OneHealth LA-MRSA surveillance will be presented.

**KL-005**

## Global epidemiology of antimicrobial resistance: Where and for what should we be looking?

Alison Mather[1]

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We have a greater understanding than ever before of the mechanisms and diversity of antimicrobial resistance (AMR), due to the enormous volumes of whole genome and metagenome sequence data available. However, simply knowing what AMR is present in a particular location or population is not enough to understand the potential risk it presents for health or onward transmission. Given the global connectivity of humans, animals, foods and environments, what risk does AMR in one location or population pose for another? At a finer resolution, given the high mobility potential of AMR genes, should we focus surveillance efforts on specific bacteria or the plasmids that carry AMR genes? In this talk I will describe a number of cases where a combination of (meta)genome and epidemiological data have shed insights into the relative risks and importance of AMR in different settings and populations.

**KL-006**

## Overview of oxazolidinone resistance in enterococci from a One Health perspective

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**Background and objectives:** Oxazolidinones (linezolid and tedizolid) are last-resort antimicrobial agents used to treat severe human infections caused by MDR Gram-positive bacteria. They bind to the peptidyl transferase centre of the bacterial ribosome inhibiting protein synthesis. Although the majority of Gram-positive bacteria remains susceptible to oxazolidinones, resistant isolates are increasingly reported worldwide. Besides ribosomal mutations, the acquisition of linezolid resistance genes (cfr and cfr-like, optrA and poxtA), often found on mobile genetic elements [such as conjugative plasmids, transposons, integrative and conjugative elements (ICEs), prophages and translocatable units], plays a key role in the spread of oxazolidinone resistance [1,2].

**Methods:** Although oxazolidinones are approved exclusively for human use, bacterial strains carrying linezolid resistance genes were detected, besides in humans, in livestock as well as in wildlife and the environment. Enterococci are widely distributed in nature and their occurrence, prevalence, and persistence across the One Health continuum make them an ideal candidate to examine antimicrobial resistance from a One Health perspective [3].

**Results:** Phylogenetic analyses and the characterization of the main genetic elements responsible for the spread of oxazolidinone resistance were addressed using the Whole Genome Sequencing approach. The occurrence of identical or closely related conjugative or mobilizable plasmids in enterococci from animals, humans and freshwater, confirms that the same genetic elements circulate in the enterococcal population and emphasizes the key role played by plasmids in the spread of linezolid resistance in this bacterial genus. The global spread of the linezolid-resistant Enterococcus faecalis ST476 clonal lineage carrying the optrA gene has also been explored [4].

**Conclusions:** The "One Health" perspective highlights the importance of surveillance systems for monitoring antibiotic resistance not only in the clinical settings but also in the animals and environmental where several relevant reservoirs of antibiotic resistance have been identified. An "One Health" approach can effectively mitigate the emergence and dissemination of antibiotic-resistant bacteria ensuring the continued effectiveness of last-resort drugs, such as oxazolidinones, for the well-being of both current and future generations.

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## KL-007

# A One Health-Perspective on Bacteriophages as novel cross-sectoral antimicrobials: Opportunities and challenges

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Antimicrobial resistance (AMR) poses a serious global threat, reducing our ability to treat bacterial infections in humans, animals, and the environment. It is estimated that bacterial AMR contributed to 4.95 million deaths in humans and was directly responsible for 1.27 million deaths globally in 2019. Addressing AMR in a multifaceted One Health approach takes into account the interconnectedness of human, animal, and environmental health and underscores the importance of intersectoral collaboration in the fight against AMR. Within the framework of various approaches against AMR, novel antimicrobials are urgently needed. However, development and approval are difficult and expensive. The World Health Organization (WHO) defines four innovation criteria for novel antimicrobials: new chemical class, new target, new mode of action, and absence of cross-resistance to traditional antibiotics. Bacteriophages (phages) can meet all four criteria. They are viruses that selectively infect and kill bacterial cells. They can be used alone or in combination with other treatments and serve as a toolbox for antimicrobial enzymes. They are the most abundant naturally occurring entities and can be isolated from all bacterial habitats. Consistently, they play a crucial role in regulating bacterial populations and shaping microbial ecosystems. They have been used against bacterial infections in countless cases in Eastern countries since they were discovered more than a century ago. They can be a valuable tool to address the challenge of AMR across sectors, ranging from therapeutic use in humans and animals to reduced antibiotic use in agriculture. The German Center for Infection Research (DZIF) promotes the best possible implementation of bacteriophage research, development, and therapy in Germany and provides a platform for interdisciplinary exchange (https://tinyurl.com/22m8cb95). However, the different genomic properties and self-

replicating nature of phages make their regulatory approval as antimicrobials challenging. A novel EMA (European Medicines Agency) Guideline on phage approval for veterinary applications has paved the way for future bacteriophage-based therapeutics in this sector (https://tinyurl.com/4b3mpstf). In human medicine, phages are currently used on compassionate grounds, in life-threatening situations, when all other treatments have been exhausted. However, a concept paper on the development and manufacture of phage therapeutics for human medicine is under development (https://tinyurl.com/bdf9xc5k), and clinical trials are running.

Phage application in humans, animals, food, and against plant pathogens is being intensively investigated worldwide. Several studies have underlined their efficacy against important pathogens like *Escherichia coli* and *Salmonella enterica*. Phages can reduce antimicrobial use in veterinary medicine and can be used alone or in combination with antibiotics to treat multidrug-resistant bacteria in humans and animals. Furthermore, they can contribute to safer food products, preventing the spread of foodborne zoonotic pathogens. However, further evidence supporting efficacy, safety, and feasibility is needed. In veterinary medicine, current research focuses on improved formulations and efficacy assessment of bacteriophages against different veterinary and zoonotic pathogens. For livestock farming, multiple-hurdle approaches have been investigated against the foodborne pathogen *Campylobacter* spp., combining organic acids, phages, and curcumin as well as phages and common antimicrobials. While encouraging results were received in animal experiments, successful application in commercial settings seems to be challenging due to various factors, including the introduction of novel, insusceptible bacterial strains (https://tinyurl.com/3hw87sec). Further studies on the mechanisms of efficient phage application against specific veterinary pathogens as well as their interactions and synergies with other treatment options are urgently needed to improve and implement their therapeutic use.

# Oral Presentation

## *Session 1 - Novel methods and tools dedicated to antimicrobial resistance (detection, diagnostics, surveillance)*

### OP-001

### Development and evaluation of a harmonized antimicrobial susceptibility testing method for porcine *Mycoplasma hyorhinis*

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**Background and objectives:** *Mycoplasma* (*M.*) *hyorhinis* is an ubiquitously occurring porcine pathogen. Depending on the pigs age at the time of infection, variable pathology can be observed, generally accompanied by great economic losses. Before treating bacterial infections with antimicrobial agents, antimicrobial susceptibility testing (AST) should be carried out in a standardized manner. However, this requires the availability of a standardized AST method. Organizations, such as the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST), provide standardized protocols for a range of fastidious bacteria but not for *M. hyorhinis* so far.

**Methods:** A broth microdilution method for testing *M. hyorhinis* was developed using a modified Friis broth devoid of antimicrobial or otherwise bacterial growth-inhibiting agents as the test medium and evaluated for standardization. In addition, the suitability of the method was evaluated via variation of the individual ingredients of the modified Friis broth by either using different batches or choosing other distributors. The type strain *M. hyorhinis* DSM 25591 (ATCC 17981) and six field isolates were chosen to develop the method. In a second step, we evaluated this broth microdilution method with 37 *M. hyorhinis* field isolates and tested their susceptibility towards the following antimicrobial agents: doxycycline, enrofloxacin, erythromycin, florfenicol, gentamicin, marbofloxacin, tetracycline, tiamulin, tilmicosin, tulathromycin, and tylosin. For this, the CLSI-approved quality control (QC) strains *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 served as provisional QC strains. The type strain *M. hyorhinis* DSM 25591 was also repeatedly tested, to assess its suitability as a potential future QC strain.

**Results:** The modified Friis broth proved to be a suitable test medium for AST of *M. hyorhinis*. During the method development as well as the batch testing of each ingredient, the *M. hyorhinis* type strain and six field isolates gave repeatable and reliable results [1]. The tested field isolates showed unimodal (gentamicin, enrofloxacin, marbofloxacin, florfenicol, erythromycin, tiamulin, doxycycline, tetracycline), bimodal (clindamycin, tilmicosin) or multimodal (tulathromycin, tylosin) distributions of MIC values. For macrolides and lincosamides, elevated MIC values could be observed for almost half of the tested field isolates, suggesting a reduced susceptibility of these *M. hyorhinis* isolates towards these substances. The type strain *M. hyorhinis* DSM 25591 yielded comparable results [2].

**Conclusions:** The presented method proved reliable and conferred repeatable results. Using established QC strains facilitated a controlled establishment of the method. Based on the robust results obtained with *M. hyorhinis* DSM 25591, we propose to implement this type strain as quality control. With this newly developed harmonized method, we aim to provide an improved AST method for *M. hyorhinis* that may be used in diagnostic laboratories and for monitoring purposes allowing for a better comparability of AST results.

**References**

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## OP-002

# Shotgun metagenomic sequencing uncovers antimicrobial resistance-encoding genes (ARG) in Irish red foxes (*Vulpes vulpes*) and European badgers (*Meles meles*)

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**Background and objectives:** Wildlife are considered reservoirs and disseminators of antimicrobial resistance-encoding genes (AMG) in the environment. However, the role of species' ecology and movement in the diversity, evolution and transmission of AMR across ecosystems remains understudied [1]. Moreover, currently in Ireland and elsewhere in Europe, there is no routine monitoring of wildlife for AMGs. Red fox (*Vulpes vulpes*) and European badger (*Meles meles*) are of interest as a potential reservoir of environmental AMG due to their behaviour and proximity to agricultural and anthropogenic landscapes. The aim of this study was to assess antimicrobial resistant (AMR) determinants and microbiota in the faeces of rural Irish foxes and badgers using shotgun metagenomics and compare these results to that of previous phenotypic antimicrobial susceptibility testing (AST).

**Methods:** To identify ARGs and microbiotal community of the animals' gut microbiomes, genomic DNA (gDNA) was purified from faecal samples (n=47) of red foxes (n=6) and European badgers (n=41) and subjected to shotgun metagenomic sequencing using long-read sequencing. Raw reads assessed for quality using NanoPlot, were assembled with Flye, and the resulting contigs were indexed using BWA. Contigs were then screened for ARGs using KMA, ResFinder, and ABRicate. Taxonomic classifications were investigated using Kraken2. Following these initial steps, alignment and assembly of metagenome-assembled genomes (MAGs) was conducted using Minimap2 and MetaBAT2, respectively. Additionally, of these 47 samples, 39 previously presented with phenotypic resistance by standardised AST [2]. These results were compared against the results of the metagenomic analyses.

**Results:** Preliminary bioinformatic analyses detected some ARGs that corresponded to the resistance profiles determined by AST. However, numerous additional ARGs were also detected in the metagenomic sequences that were not reflected in the phenotypic screening of these samples. Further bioinformatic processing and downstream analyses are currently ongoing involving conducting comprehensive exploration of microbial community composition, plasmid transfer and metabolic pathways.

**Conclusions:** The preliminary results of this study confirm presence of AMGs in red foxes and European badgers in Ireland. The differing depth of information garnered from the metagenomic analyses *versus* the phenotypic results suggest differences in testing scope and sensitivity and serves to highlight the importance of developing standardised methods for surveillance and reporting of AMGs in wildlife and the environment.

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**OP-003**

# Rapid chute-side antibiotic resistance detection tools to improve antimicrobial stewardship and risk management while controlling bovine respiratory disease

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**Background and objectives:** A chute-side lateral-flow immunoassay (LFIA) that can speed the detection of antimicrobial resistance (AMR) elements prevalent in bacterial pathogens would significantly improve the management of bovine respiratory disease (BRD). By optimizing risk management for both respiratory and enteric bacterial AMR, livestock can remain healthy while ensuring the safety of the food supply. The objective of the study was to assess the sensitivity (Se) and specificity (Sp) of two rapid diagnostic tools targeting (1) extended-spectrum beta-lactamase (CTX-M)/mobilized AmpC (CMY-2) proteins (duplexed) and (2) overall 3rd generation cephalosporin (cefotaximase) activity in the feces of feeder cattle.

**Methods:** The objective was achieved using Bayesian latent-class models (BLCM) for novel LFIA comparisons against conventional microbiological endpoints. The analyses focused on two populations of fecal bacteria, differing in beta-lactamase prevalence due to pre- and post-antibiotic treatment. A total of 400 beef cattle were included in a randomized field trial across two major U.S. beef cattle feed yards. On Day 0, 200 individual fecal samples were collected per rectum on each operation, following which each animal was assigned one of four antibiotic treatments (macrolide, fluoroquinolone, phenicol, or third-generation cephalosporin (or no treatment in one feed yard)) following the single-dose labeled regimen for BRD control (i.e., 50 cattle per group). After seven days, 200 post-treatment fecal samples were collected and matched on animal IDs. These samples were examined for AMR through microbiological analysis via spiral plating on selective media (MacConkey agar with 4 mg/L of ceftriaxone). The outcomes of this study were compared with those obtained from the duplexed, the cefotaximase activity, and a sample subset via an improved version of the cefotaximase activity DetecTooLs (NG Biotech, Guipry, France).

**Results:** Antibiotic metaphylaxis for BRD resulted in an increase in the prevalence of beta-lactam resistance elements and enzymatic activity on Day 7 compared to pre-treatment on Day 0. Based on this difference, BLCM analysis indicated an increasing Se of the duplexed assay, rising from 81% at $10^2$ CFU/g feces to 99% at $10^5$ CFU/g. Meanwhile, assay Sp decreased from 93% to 89% over the same range of bacterial counts. The cefotaximase activity assay initially showed lower Se across the same range of coliform CFU (40-75%); however, Sp remained constant at 100% across the same range. The sample subset analysis of the improved version of the cefotaximase activity assay showed a markedly higher sensitivity than the first version. Work with this latter assay is ongoing. Interestingly, macrolide use during metaphylaxis exerted very little selection pressure on beta-lactamase outcomes

compared to non-treated controls. In contrast, phenicols led to the highest levels of selection for beta-lactam resistance, even when compared to the direct selection of a 3rd generation cephalosporin.

**Conclusions:** The results indicate that pen-side rapid assays can inform AMR stewardship decisions regarding BRD metaphylaxis. Implementing these tools in the field would likely require additional equipment and staff training. This preliminary study emphasized enteric bacteria as the component of food safety in livestock production. Our current research focuses on these chute-side assays on respiratory pathogens and macrolide resistance.

**OP-004**

## RamanBioAssay™: Rapid antibiotic susceptibility testing of *Escherichia coli* isolates from pig farms

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**Background and objectives:** Fluoroquinolones and cephalosporins are frequently used antibiotics to which *Escherichia coli* is becoming increasingly resistant, posing a major public health challenge. This study employs Raman spectroscopy (RamanBioAssay™ [1]) to rapidly determine the antibiotic susceptibility of *E. coli* isolates, which originate from the environment of pig farms and contaminated houseflies, both potential transmission routes to humans.

**Methods:** Standard antibiotic susceptibility testing assesses bacterial growth or inhibition in the presence of an antimicrobial agents. Conversely, the RamanBioAssay™ is based on the specific spectral signals that bacteria exhibit when being exposed to anti-infective agents. The identification of resistance patterns from spectral signatures is facilitated by the distinct cellular responses that anti-infective agents induce in susceptible strains compared to resistant strains.

**Results:** This method enables the detection of antibiotic effects, particularly for ciprofloxacin and cefotaxime, within 90 minutes of exposure, allowing for the rapid differentiation between sensitive and resistant bacteria.

**Conclusions:** By providing a fast and effective approach to identifying antibiotic resistance, this method could improve diagnostic accuracy and accelerate response times in various settings.

[1] Kirchhoff, J. et al., Analytical Chemistry 2018 90 (3), 1811-1818

**Acknowledgements**

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**Figure**

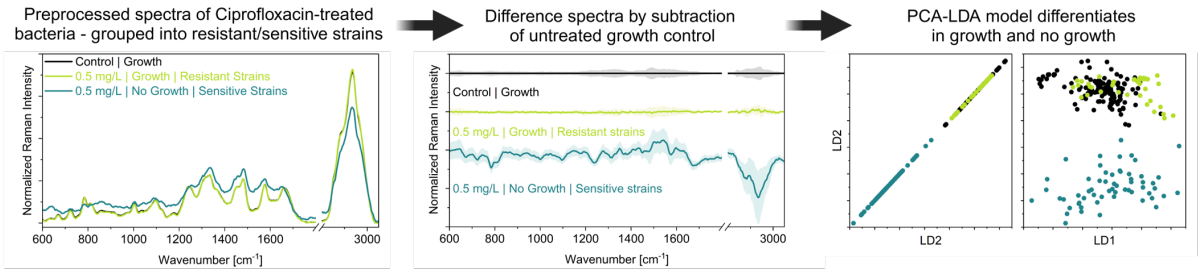


**Figure 1:** Accelerating diagnostics: from spectra, taken 90 minutes after bacteria-antibiotic interaction, to model-based differentiation.

## OP-005

# Development and Validation of a Targeted Next-Generation Sequencing (tNGS) Assay for Concurrent Detection of Pathogens and Antimicrobial Resistance Genes in Swine Clinical Specimens

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**Background and objectives:** Swine farms worldwide are increasingly challenged by complex co-infections and escalating antimicrobial resistance (AMR), necessitating rapid and comprehensive diagnostic approaches for effective disease management. This study compares the performance of a novel tNGS platform with conventional methods (qPCR, bacterial culture, and MIC testing) in detecting pathogens and AMR genes from 367 clinical specimens derived from diseased pigs, highlighting its value in One Health-oriented antimicrobial resistance surveillance.

**Methods:** Clinical specimens were categorized into three distinct diagnostic groups: Serum analysis cohort (n=36): Viral pathogens identified via qPCR. Swab validation cohort (n=33): Nasopharyngeal swabs analyzed through bacterial culture followed by MIC determination, with simultaneous tNGS analysis conducted for cross-validation. Field surveillance cohort (n=298): Pathogen genomes and AMR genes enriched via ultra-multiplex PCR, followed by high-throughput sequencing and bioinformatic characterization of polymicrobial infection dynamics.

**Results:** Analysis of 298 clinical specimens revealed a predominant prevalence of polybacterial-viral-mycoplasmal co-colonization (251/298, 84.2%). tNGS platform demonstrated concurrent pathogen identification and AMR determinant profiling across 69 clinical specimens. Key findings included: Serum analysis (36) detected PRV and PCV2/3 as dominant viruses, with *S. suis* and *Mycoplasma* co-infections observed in 8 samples. Nasopharyngeal swabs exhibited 100% polymicrobial colonization (33/33), primarily *S. suis* and *H. parasuis*, with co-detection of *Pm* and *Mhr/Mhp*. tNGS excluded *Toxoplasma gondii* (consistent with qPCR) while identifying pathogens undetected by conventional

methods. AMR profiling identified high-abundance resistance genes targeting aminoglycosides, β-lactams, chloramphenicol, tetracyclines. Sulfonamides and MLSB resistance genes were less prevalent, while fluoroquinolone and vancomycin resistance genes remained undetected. MIC results for *S. suis* and *H. parasuis* isolates strongly correlated with tNGS-derived resistance profiles.

**Conclusions:** This study establishes a novel tNGS-based diagnostic approach for concurrent detection of pathogens and AMR genes in swine diseases. The method demonstrates superior accuracy in characterizing complex co-infections and resistance patterns compared to conventional techniques, providing valuable guidance for clinical antimicrobial selection and advancing One Health surveillance strategies.

## *Session 2 - Roles of the environment in resistance evolution and transmission*

### OP-006

# Redox Fluctuation Frequency Influences Antibacterial Resistance Evolution through ROS-Induced DNA Damage and Repair

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**Background and objectives:** While antibiotic exposure is traditionally considered the primary driver of resistance evolution, emerging evidence suggests that environmental factors, such as climate warming [1], and oxidative stress [2] may play a crucial role. Environmental redox fluctuations vary significantly across ecosystems, from diurnal cycles in shallow waters to seasonal shifts in stratified water bodies, with each pattern potentially imposing distinct selection pressures on microbial communities. This study investigates how varying redox oscillation frequencies drive antibiotic resistance evolution through reactive oxygen species (ROS)-mediated DNA damage and repair mechanisms.

**Methods:** We analyzed metagenomic and metatranscriptomic data from a redox oscillation study [3] where river sediment microbial communities were exposed to high-frequency (HF, ~0.5-day), medium-frequency (MF, ~2-day), and low-frequency (LF, ~8-day) oxic/anoxic transitions. Following Illumina NextSeq 500 (2 × 151 bp) sequencing, metagenomes were assembled using SqueezeMeta v1.6.0, filtered for redundancy (cd-hit), and annotated against GenBank, KEGG, and CARD databases. Metatranscriptomic reads (Illumina MiSeq, 2 × 150 bp) were mapped to these assemblies using Bowtie2 to assess ARG expression across the different redox fluctuation conditions.

**Results:** HF redox fluctuations significantly increased the expression of ARGs, particularly β-lactamases (*e.g.*, *penP*, *oxa*), and efflux pumps (*e.g.*, *MexW*, *rsmA*, *YajC*), while ARG potential and transcription were markedly lower in LF conditions. We observed significant upregulation of electron transport chain genes (*e.g.*, *sdhA*, *petA*, *CYC*) in HF, suggesting elevated ROS production. This elevated ROS production correlated with increased expression of oxidative stress response (*e.g.*, *oxyR*, *sodAB*, *katE*, *gpx*, *trxB*). The TC-NER DNA repair pathway was strongly induced in HF, with increased expression of *mfd*, *uvrA*, and RNA polymerase subunits (*rpoA*, *rpoB*, *rpoC*), highlighting an enhanced DNA damage response. Conversely, LF exhibited minimal stress response and repair activity, suggesting lower oxidative stress pressure. These findings demonstrate that high-frequency redox fluctuations accelerate antibiotic resistance evolution via ROS-induced mutagenesis and transcription-coupled repair mechanisms.

**Conclusions: Conclusions:** Environmental redox fluctuations promote antibiotic resistance evolution by inducing ROS-driven genetic variability, enriching resistant mutants even without antibiotic

selection. Our novel findings identify the TC-NER pathway, particularly the mfd repair protein, as a key mechanism linking environmental stressors to resistance development. This work expands our understanding of how redox dynamics in aquatic systems may contribute to antibiotic resistance persistence.

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[1] Manna B, Jay E, Zhang W, Zhou X, Lyu B, Thomas GM, Singhal N (2024). *Water* 16:2655.

[2] Manna B, Zhou X, Singhal N (2025). *Water Research* 277:123366.

[3] Li S, Mosier D, Dong X et al. (2023). *ISME J* 17:2047-2057.

## OP-007

# Assessing Resistome Risk in Pathogen Transmission Across the Dairy Production Environment

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**Background and objectives:** Pathogens which commonly harbour antimicrobial resistance (AMR) genes, can move seamlessly between dairy cattle, manure, and the surrounding environment, posing risks to both livestock and human health. While research has extensively explored interventions to curb their impact on dairy health, a critical gap remains in understanding how manure and soil management shape pathogen persistence and AMR transmission across the dairy production environment. Understanding these dynamics is essential for developing sustainable strategies that limit the spread of antibiotic resistance. Here, we investigated whether soil management practices could be harnessed to reduce the abundance, diversity, and spread of AMR genes from manure fertilizers using a large-scale field study at an integrated cropping system trial (WI, USA).

**Methods:** Soil cores were collected pre- and post-manure from 4 repeated blocks of continuous corn (CC), conventional forage (CF), organic forage (OF), and rotational grazing (RG) plots. Dairy manure was collected from application tankers and a storage lagoon. We applied metagenomic shotgun sequencing using an Illumina NovaSeq with 2x150 paired-end chemistry, targeting 80 million reads/sample. Raw reads were trimmed and aligned using AMR++ v 3.0 for taxonomy and ARG classification. After read assembly, MetaCompare 2.0 was used to classify mobile genetic elements (MGEs) and generate human health and ecological resistome risk (RR) scores.

**Results:** Bacterial alpha diversity was significantly ($p<0.05$) different across all cropping systems, except conventional and organic forage, which were significantly higher especially post-manure application. Overall, soil harboured a more diverse resistome relative to manure, while MGEs were more prevalent in manure. These results indicate that manure poses a higher risk of transmitting ARGs due to its abundance of MGEs and ARG-MGE combinations. Manure contained 186 unique AMR genes, dominated by tetracycline (30.1%) and macrolides (25.9%). Soil, on the other hand, contained 456 unique AMR genes and resistance to rifampin (15.9%), macrolides (14.2%) and beta-lactams (11.8%) were most abundant. Soil management had minor effects on resistome profiles or

transmission risks, but forage practices had the lowest risks overall: CF (4.16), OF (4.19), RG, (4.38), and CC (4.57).

**Conclusions:** These findings highlight manure as a critical control point for reducing the spread of AMR genes and underscore manure amended soil as a potential reservoir for diverse resistomes. Soil management played a minor role in mitigating AMR spread; however, forage soil management practices may warrant further investigation as a potential strategy to reduce the hazards associated with manure fertilizers, given their lower resistome risk.

## OP-008

# Metabolism-driven resistance: uncovering a novel glyphosate survival strategy in *Klebsiella*?

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*[3]University College Dublin, School of Biology and Environmental Science, Dublin, Ireland*

**Background and objectives:** The shikimic acid metabolic pathway supports the biosynthesis of aromatic amino acids in plants and microorganisms. Glyphosate (GLP) is a competitive inhibitor of this pathway and a key ingredient in broad-spectrum herbicides such as RoundUp®. *Klebsiella pneumoniae* is associated with plant roots and has recently been identified as a hitherto unrecognised zoonotic bacterium of importance to *One health*, often characterised by its hypervirulence and multidrug resistance (MDR) phenotypes in clinical settings. Intriguingly, transient exposure to GLP in the environment could lead to changes in susceptibility to one or more antimicrobial compounds in root-associated *K. pneumoniae*, and these changes could lead to the emergence of untreatable acquired infections that have an impact on public health[1].

**Methods:** A collection of five *Klebsiella* isolates was included in the study, consisting of the type strain *K. pneumoniae* MGH 78578, two clinical *K. pneumoniae* isolates, as well as an environmental (*K. variicola*) and an animal (*K. variicola*) isolate. The minimum inhibitory concentration (MIC) to a panel of antimicrobial agents was determined using Sensititre plates in the presence and absence of a sub-inhibitory GLP concentration (0.25 X MIC). A laboratory generated GLP mutant (expressing 2 X MIC) was selected, and the whole bacterial genome sequence determined for the entire collection. Further phenotype microarray (PM) experiments were carried out (in the absence and presence of 0.25 X MIC GLP) to explore the metabolic impact of herbicide selection.

**Results:** Sublethal exposure to GLP uncovered phenotypic resistance mechanisms to several critically important antimicrobial (CIA) compounds including imipenem, levofloxacin and tigecycline in all study isolates. Genome analysis highlighted roles for genes regulating the AcrAB-TolC efflux pump, alterations in outer membrane porins, along with target gene mutations in *gyrA* and *parC*, and the *tetA* and *tetX* genes, underpinning the observed changes in phenotypic resistance. The GLP mutant created for the *K. pneumoniae* MGH78578 evolved two interesting locus variants that contributed to a change in its sequence type; firstly in the *mdh* gene involved in the conversion of malate to oxaloacetate in the TCA cycle, and secondly two other mutations were observed in *phoE,* an outer membrane porin involved in phosphate transport.

Separately, PM experiments confirmed that under sublethal GLP exposure, *Klebsiella* channelled intermediates through the TCA cycle with limited flux through glycolysis. The GLP mutant selected also contained one or more SNPs in every gene encoding enzymes of the TCA cycle.

**Conclusions:** Previously reported mechanisms of antibacterial resistance to GLP have not implicated the central metabolic pathways, and this could be of importance with the growing interest in the role of

bacterial metabolism in antimicrobial resistance[2]. This study underlines the potential public health risks of environmental herbicide exposure. Furthermore, it demonstrates that sub-inhibitory GLP concentrations can extend the repertoire of antimicrobial resistance phenotypes observed, unmasking resistance to CIA type compounds. Continuing work in this laboratory is focused on attempting to understand the potential cross-resistance mechanisms at play in these *Klebsiella* species.

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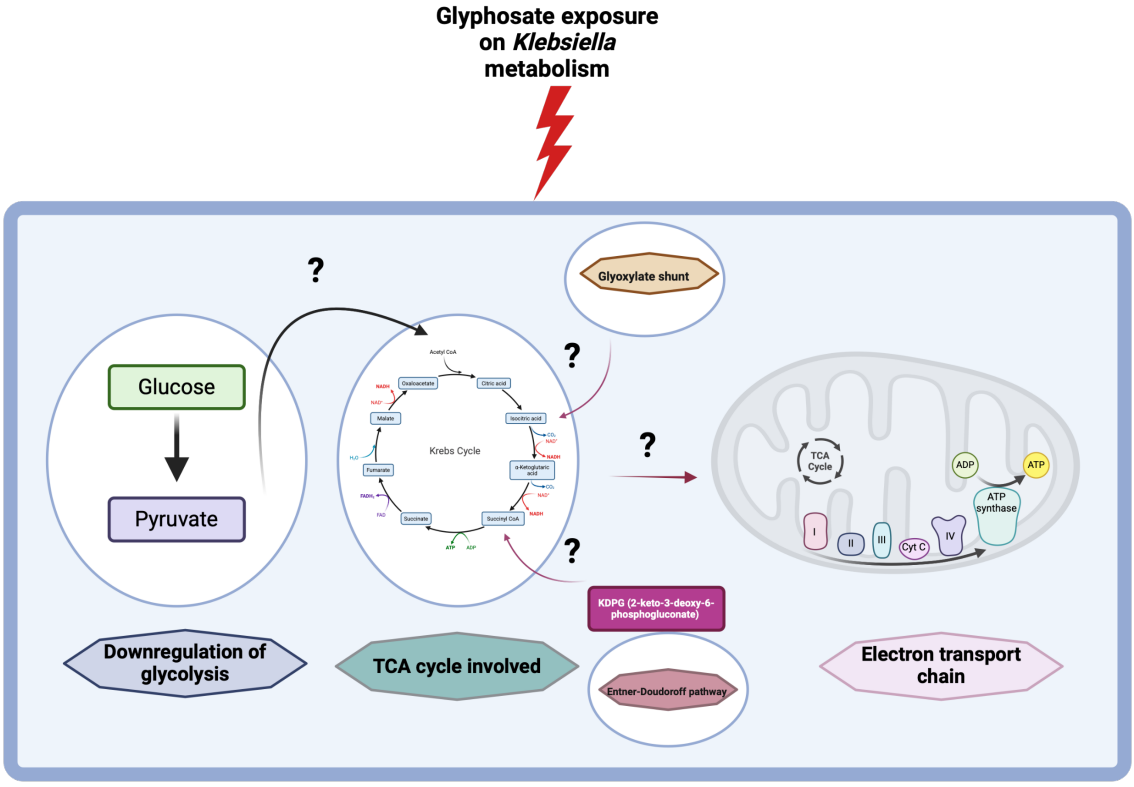
[2] Ahmad, M., S. V. Aduru, R. P. Smith, Z. Zhao & A. J. Lopatkin (2025) The role of bacterial metabolism in antimicrobial resistance. Nature Reviews Microbiology.

**Figure**




**OP-009**

## Prophage induction by non-antibiotic pollutants promotes transformation of released antibiotic resistance genes from cell lysis

Ji Lu[1], Jianhua Guo[1]

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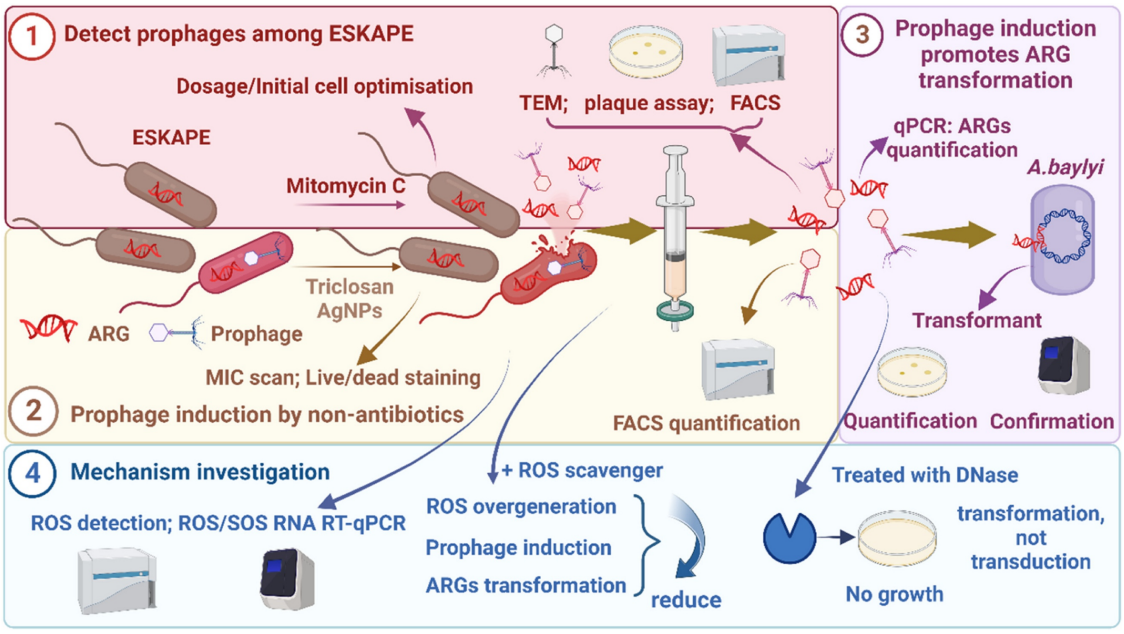
**Background and objectives:** Prophages are common among bacterial species, including strains carrying antibiotic resistance genes (ARGs). Prophage induction can be triggered by the SOS response to stressors, leading to cell lysis and the subsequent release of ARGs. Environment pollutants are known to cause stress responses among environmental bacteria. To date, the extent of the risk posed by non-antibiotic pollutants in triggering prophage induction among ARG-carrying bacteria, as well as the subsequent spread of ARGs after cell lysis, remains unknown.

**Methods:** We combined plaque assays, flow cytometry, and transmission electron microscopy to identify prophages and ARGs carried by clinical ESKAPE isolates (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *A. baumannii*). We tested whether non-antibiotic pollutants triclosan and silver nanoparticles could trigger prophage induction among those ESKAPE isolates and explored the underlying mechanism by measuring reactive oxygen species generation and transcriptional changes in the SOS response. Finally, we quantified ARG release following pollutant-induced prophage induction and assessed the subsequent risk of ARG transformation to *Acinetobacter baylyi* from cell lysis.

**Results:** Environmental pollutants triclosan and silver nanoparticles, at environmentally relevant and commercial concentrations, enhanced prophage induction by up to 5.6 times in ESKAPE isolates. Transmission electron microscopy imaging and plaque assays confirmed the production of infectious phage particles under non-antibiotic pollutants-mediated prophage induction. In addition, the rate of ARG transformation to *A. baylyi* significantly increased for two-fold after the release of extracellular ARGs from prophage induction-mediated cell lysis. The mechanism of non-antibiotic pollutants-mediated prophage induction is primarily associated with excessive oxidative stress, which provokes the SOS response.

**Conclusions:** Environmental pollutants triclosan and silver nanoparticles promote ARG transformation from ESKAPE isolates by triggering prophage induction, primarily through oxidative stress–induced SOS response. The cross-species transformation of ARGs resulting from prophage-induced host cell lysis, as identified in this study, advances our ecological understanding of the environmental spread of antimicrobial resistance in diverse bacterial species. Considering the prevalence of prophages identified in clinically important antibiotic-resistant pathogens and even a broader range of bacterial species, our findings raise concerns regarding the potential antibiotic-like roles of non-antibiotics. These findings highlight the role of non-antibiotic pollutants in facilitating ARG dissemination via prophage activation.

**Figure**



**OP-010**

## Investigating the Presence and Profile of Antimicrobial Resistance Determinants in Harvested Rainwater

Michael Arthur[1,2], Elena-Alexandra Alexa[2], Amy McKenna[1], Michael Gaffney[1], Jesus Frias[2], Catherine Burgess[1]

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**Background and objectives:** Roof-harvested rainwater is increasingly used for irrigation due to water scarcity and the need for more sustainable agricultural practices. However, there are concerns regarding microbial contamination, particularly the presence of antimicrobial resistant organisms (AROs) and genes (ARGs), as these pose significant risks to food safety and public health. Rainwater can accumulate environmental contaminants, including AROs from atmospheric deposition, bird droppings, and bioaerosols, which may introduce resistant pathogens into the food chain. This study investigated the prevalence of ARGs in roof-harvested rainwater collected from a number of different roof types in Ireland.

**Methods:** A total of 84 rainwater samples were collected over two sampling periods, each spanning six weeks. Samples were filtered using 0.45 µm membrane filters, and DNA was extracted accordingly. Shotgun metagenomic sequencing was performed, followed by metagenomic analysis to identify ARGs and their relative abundance across sampling weeks.

**Results:** Metagenomic analysis revealed the presence of multiple ARG families in the rainwater samples. Genes encoding resistance mechanisms to beta-lactams, aminoglycosides, tetracyclines, and multidrug were detected across all sites. The abundance of ARGs varied over the sampling weeks, with notable fluctuations in resistance gene counts. Some sites exhibited higher cumulative ARG prevalence, suggesting site-specific variations in contamination sources. The overall data indicate that roof-harvested rainwater can serve as a reservoir for AMR determinants, with potential implications for its use in irrigation.

**Conclusions:** The detection of ARGs in roof-harvested rainwater highlights a critical public health concern. Irrigation with contaminated water may facilitate the transfer of resistance genes to soil and fresh produce, increasing the risk of antimicrobial-resistant infections through food consumption. These findings underscore the need for routine monitoring of rainwater quality and the implementation of appropriate treatment measures to ensure its safe use in agriculture.

**OP-011**

## Clinically prevalent transposons contribute to *erm* gene dissemination in soil with long-term erythromycin at environmentally relevant concentrations

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**Background and objectives:** Clinically relevant antibiotic resistance genes (ARGs) or their ancestral genes are widespread in the natural environment at ultralow abundance, which has been widely recognized as the origin and contributor of clinical resistance crisis. However, the prevalence of these ARGs in the environment remains unclear, partly due to the limited understanding of how

environmental microbiomes respond to long-term antibiotic contamination at environmentally relevant concentrations.

**Methods:** We conducted a consecutive five-year field exposure experiment involving 5 to 20 μg·kg-1 of erythromycin, the first-generation macrolide. Metagenomic sequencing together with ARG quantification, sequence assembly and binning were conducted.

**Results:** The primary clinically relevant macrolide resistance genes, 23S rRNA methyltransferase genes (*erm* genes), were initially rare but gradually enriched, exhibiting a 37.8-fold increase after five years. By contrast, the enrichment of macrolide efflux pump genes and inactivation genes was no more than 2.3-fold. Among diverse mobile genetic elements, transposase gene *tnpA* exhibited strong association with the horizontal transfer of *erm* genes during long-term erythromycin exposure. Based on genetic and statistical evidence, we found that the *erm* genes in the environment were located on mobile transposable elements Tn*554* and Tn*551*, which were clinically prevalent gene clusters in the pathogens-*Enterococcus* and *Staphylococcus*. At the same time, the environmental host bacteria of *erm* genes were identified as Bacilli, which was phylogenetically close to the pathogenetic host bacteria of *erm* genes.

**Conclusions:** Our findings demonstrate the historical contribution of erythromycin-contaminated environmental microbiomes to clinical macrolide resistance, and suggest to rethink the lasting evolution and dissemination of clinically relevant ARGs under environmentally realistic concentration of antibiotics.

## OP-012

# Vancomycin heteroresistance in a vancomycin variable *Enterococcus faecium* from marine sediment

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**Background and objectives:** Vancomycin-variable enterococci (VVE) have emerged as hospital-acquired pathogens alongside vancomycin-resistant enterococci. VVE strains appear susceptible to vancomycin but carry a silent van operon, enabling reversion to resistance and potential treatment failure. This study investigates the phenotypic and genetic features of *E. faecium* JSEG15 environmental VVE strain.

**Methods:** Whole-genome sequencing (WGS) was performed to genetically characterize the parental strain and three revertants selected through vancomycin exposure. RT-qPCR was used to assess the *vanA* copy number and expression levels between the VVE strain and its revertants. Vancomycin heteroresistance was evalueted by E-test and population analysis profiling.

**Results:** The *E. faecium* JSEG15 strain, belonging to ST80 (one of the dominant *E. faecium* sequence type associated with nosocomial infections), was susceptible to vancomycin despite carrying a Tn*1546*-like transposon on a 36,019 bp plasmid. Gradual exposure to increasing vancomycin concentrations led to the selection of three resistant revertants with growing levels of resistance. All revertants harbored a deleted Tn*1546*-like element on a 34,728 bp plasmid (Figure 1A). Notably, JSEG15-rev2 and JSEG15-rev3 acquired an additional chromosomal copy of the *vanHAX* cluster, along with increased *vanA* copy number and expression, explaining their higher resistance levels. JSEG15 and JSEG15-rev1 showed inducible resistance to vancomycin whereas JSEG15-rev2 and JSEG15-rev3 exhibited constitutive *vanA* expression. Only JSEG15-rev3 was able to transfer

resistance by *in vitro* conjugation. Both the parental strain and all revertants exhibited vancomycin heteroresistance, with revertants showing a higher frequency of resistant subpopulations (Figure 1B).

**Conclusions:** This study highlights that behind a VVE strain often lies an heteroresistant VRE which can evolve into fully resistant strains upon vancomycin exposure. To the best of our knowledge this is the first report of a heteroresistant VVE strain from an aquatic environment, underscoring the potential role of environmental reservoirs in the emergence of vancomycin resistance and the need for environmental monitoring.

**Figure**

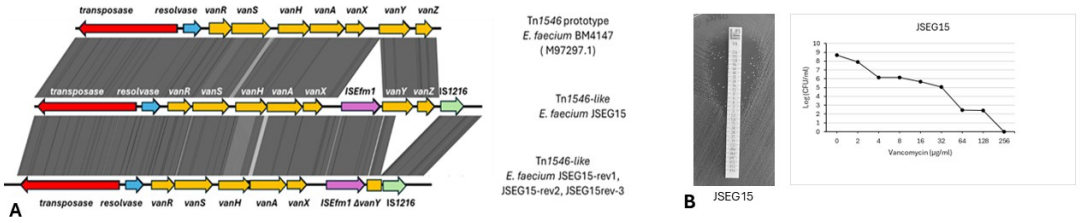


Figure 1. A) Linear map of the Tn*1546* prototype of *E. faecium* BM4147 (accession no. M97297.1) in comparison with Tn*1546*-like transposons of *E. faecium* JSEG15, JSEG15-rev1, JSEG15-rev2, and JSEG15-rev3 using Easyfig tool. B) *E. faecium* JSEG15 vancomycin heteroresistance by E-test and PAP analysis.

## OP-013

# Deciphering the role of hospital wastewater in antibiotic resistance within sewer systems


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**Background and objectives:** Hospital wastewater (WW) contains antibiotic-resistant bacteria (ARB) and a complex mix of chemical agents, including antibiotics (ATB). Understanding how hospital-derived microbiota and chemical compounds contribute to antimicrobial resistance (AMR) in domestic WW from households is essential. This study aimed to assess their respective roles in resistance selection within WW systems.

**Methods:** Over two years, hospital WW, domestic WW and mixed WW (combination of hospital and domestic sources) were sampled and analysed for bacterial resistance to ciprofloxacin (CIP) and cefotaxime (CTX), antibiotic resistance genes (ARGs), bacterial community composition and ATB concentrations. The SELECT method [1] was adapted to assess the risk of resistance selection in domestic WW following exposure to hospital WW chemicals. Additionally, controlled *in vitro* microcosm experiments were conducted, where domestic WW was combined with either whole hospital WW, or its chemical fraction or its microbiota. Resistance selection was evaluated through proportions of CIP- and CTX-resistant ARB, relative ARG abundance via HT-qPCR and ARG sequence variants using multiplex amplicon sequencing.

**Results:** Resistance to CIP and CTX, as well as most ARGs, were lower in domestic or mixed WW than in hospital WW, and certain ARGs (e.g., *blaNDM*, *blaVIM*) were only detected in the latter. Bacterial community composition differed significantly, with domestic and mixed WW resembling

environmental populations, while hospital WW was more human microbiota-associated. Predicted risk analysis indicated that most ATBs in hospital WW posed a moderate-to-high risk for resistance selection. The adapted SELECT method showed potential resistance selection in domestic WW microbiota exposed to hospital WW's chemicals fraction, and that antibiotics were not the only chemicals driving this selection. Microcosm experiments revealed that CIP- and CTX-resistant *E. coli* proportions were similar when exposed to whole HWW or its chemical fraction, but lower with microbiota alone. This suggests selective pressure from chemical exposure rather than community coalescence drove resistance for this bacterial population. During the first set of microcosm experiments, some ARGs variants were wastewater-source specific, while others were shared. Certain hospital-derived ARG variants (e.g., *blaMIR, mdtG*) persisted and coexisted alongside DWW variants, whereas others (e.g., *blaFOX*) were not maintained. These findings indicate that genes from hospital WW can persist in domestic WW.

**Conclusions:** This study highlights the complex interplay between hospital-derived ARGs and chemicals in WW. It provides insights into potential dissemination pathways and persistence patterns, improving our understanding of AMR risks in WW systems.

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## *Session 3 - Monitoring and molecular epidemiology of antimicrobial resistance*

**OP-014**

# Unraveling Antimicrobial Resistance in *Mannheimia haemolytica* Isolates from Diseased Cattle: A Phenotypic and Genotypic Analysis

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**Background and objectives:** *Mannheimia haemolytica*, an opportunistic pathogen in the upper respiratory tract of healthy cattle, can invade the lungs of immunocompromised cattle after viral infections, stress, or other risk factors, contributing to bovine respiratory disease (BRD) and enzootic pneumonia. Resistance to commonly used antimicrobials for treating BRD-associated *M. haemolytica* infections has been rising in U.S. dairy and beef cattle. We investigated the antimicrobial resistance

(AMR) phenotypic and genotypic profiles of *M. haemolytica* isolates from bovine clinical samples collected during the National Animal Health Laboratory Network (NAHLN) AMR Pilot Project (2018-2022) and employed a single nucleotide polymorphism (SNP) genotyping scheme to identify key genotypes associated with mobile genetic elements (MGEs) and AMR genes.

**Methods:** *M. haemolytica* isolates from routine diagnostic case submissions collected by NAHLN laboratories were tested for antimicrobial susceptibility (AST) using the microdilution method with CLSI veterinary breakpoints. A subset of these isolates was whole genome sequenced to identify AMR determinants and assess concordance with AST results. Subsequently, a previously established SNP genotyping scheme was used to classify *M. haemolytica* into genotypes, identifying associations with diseased cattle lungs, AMR determinants, and MGEs.

**Results:** Most isolates were pan-susceptible (67%, 1830/2743), with 33% (913/2743) resistant to at least one antimicrobial, primarily tetracycline. Among the resistant isolates, 57% (517/913) classified as multidrug-resistant (MDR). AST results and AMR genes were highly concordant (>95%) in sequenced isolates (n = 848). *M. haemolytica* isolates primarily belonged to Genotype 2 (88%, 749/848), a group previously linked to BRD, T4SS-ICE homologs, and AMR genes, consistent with our findings. Of 294 isolates harboring ≥1 AMR gene, most contained AMR genes from AMR region 2 in a T4SS-ICE (*aadA*, *ant(2'')-Ia*, *bla*OXA-2, *mph*(E), *msr*(E), and *tet*(H)), and 63 had an integrative and mobilizable element (IME) with *bla*ROB-1 and *estT*. An overall decline in *aph(3'')-Ib*, *aph(3')-Ia*, *aph(6)-Id*, and *sul2* genes within AMR region 1 was observed, which correlated with a decrease in spectinomycin resistance. Over time, the prevalence of pan-susceptible *M. haemolytica* increased.

**Conclusions:** This study highlights the prevalence of AMR in *M. haemolytica* isolates from veterinary diagnostic cases in the U.S., with significant associations between AMR genes, MGEs, and Genotype 2, commonly linked to BRD. Clinically relevant *M. haemolytica* can be genotyped with SNP-based phylogeny, providing valuable insights into strategies for mitigating BRD on farms and emphasizing the importance of monitoring resistance over time.

## OP-015

# Genetic basis of antimicrobial resistance in *Pasteurellaceae* of diseased cattle and pigs from Germany

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**Background and objectives:** *Mannheimia haemolytica* and *Pasteurella multocida* from the *Pasteurellaceae* family are central pathogens of a number of diseases, such as multifactorial respiratory diseases or mastitis, which can lead to considerable economic losses in various host species. Macrolides are frequently used for metaphylaxis and therapy, however, current data from the German national resistance monitoring GE*RM*-Vet suggest a slow increase in macrolide-resistant *M. haemolytica* and *P. multocida* from cattle. This study investigates the genetic basis of macrolide resistance and further antimicrobial resistance (AMR) properties in *M. haemolytica* and *P. multocida* from diseased cattle and pigs from Germany.

**Methods:** *Pasteurellaceae* are occasionally recognized as macrolide-resistant in routine diagnostics. Seventeen such isolates from respiratory diseases included in GE*RM*-Vet were investigated (*M. haemolytica*, cattle, 2008-2020, *n* = 13/780; *P. multocida*, pigs, 2008-2021, *n* = 4/1115). In addition, further isolates from sporadic cases of bovine *P. multocida* mastitis were considered (2021-2023, *n* =

8). Antimicrobial susceptibility testing was carried out via broth microdilution according to CLSI recommendations. Closed whole genome sequences were generated via hybrid assembly of Illumina MiSeq and Oxford Nanopore MinION reads. The common databases were used as reference for isolate typing and detection of AMR genes and mobile genetic elements (MGEs).

**Results:** Of 25 isolates tested, all but one isolate from mastitis showed increased minimal inhibitory concentrations of or were resistant to at least one, but mostly several of the antimicrobial agents tested. These included classes such as aminoglycosides, phenicols, penicillins, tetracyclines, macrolides and sulfonamides. In 19 isolates (respiratory disease *n* = 12, mastitis *n* = 7), integrative and conjugative elements (ICEs) were identified that conferred multidrug resistance. These ICEs, some of them novel, harbored the AMR genes *erm*(T), *lnu*(H), *esT*, *mef*(C), *mph*(G), *floR*, *catA3*, *aadA31*, *aad(3")(9)*, *aph(3')-Ia*, *aac(3)-IIa*, *strA*, *strB*, *tet*(H), *tet*(Y), and *sul2* in varying combinations. Four *M. haemolytica* also carried a 4,613-bp plasmid with the β-lactamase gene *bla*ROB-1. The typing results and the relatedness of the detected ICEs suggest that the bovine respiratory tract is also the reservoir of the mastitis-associated *P. multocida*.

**Conclusions:** The *Pasteurellaceae* investigated here showed resistance to several classes of therapeutically relevant antimicrobial agents. Various resistance-mediating MGEs, such as ICEs or plasmids, were identified, which can promote the rapid spread of AMR via horizontal gene transfer and co-selection processes. Although multidrug resistance still appears to be a comparatively rare phenomenon in bovine and porcine *Pasteurellaceae* from Germany, pathogen identification with subsequent antimicrobial susceptibility testing is strongly recommended prior to the start of antimicrobial therapy.

**OP-016**

## Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae in Pets, Humans and Their Environments in Lagos and Ibadan, Nigeria

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**Background and objectives:** Extended-spectrum beta-lactamase producing Enterobacteriaceae constitute a very important group of antimicrobial resistant bacteria associated with refractory life-threatening infections in humans and animals. Interactions between humans and pets may facilitate exchange of antimicrobial-resistant bacteria between humans and animals. This study investigated the distribution and possible exchange of ESBL-producing Enterobacteriaceae between humans, pets (dogs and cats) and their shared environment under household and veterinary clinic settings.

**Methods:** Faecal samples from humans (n=42) and dogs (n=447), cats (n=13) as well as swabs of surfaces of inanimate objects (n=82) within pet-keeping household and veterinary clinics in Lagos and Ibadan, Nigeria were examined for phenotypic detection of ESBL-producing Enterobacteriaceae. Bacterial isolation was done by selective isolation on MacConkey agar supplemented with either cefotaxime (1 mg/L) or ceftazidime (1 mg/L). Isolates were identified by cultural and biochemical characteristics and tested for antimicrobial susceptibility by disk diffusion method according to the recommendation of the Clinical and Laboratory Standards Institute. Phenotypic ESBL-production was determined by using cefotaxime/cefotaxime+clavulanic acid and ceftazidime/ceftazidime+clavulanic acid double disk combination test. All ten isolates from humans, pets and environmental sources in a

particular veterinary clinic in Lagos were subjected to whole genome sequencing (WGS) and sequences were analysed in order to determine the genetic relatedness among the selected isolates.

**Results:** Phenotypic ESBL-producing Enterobacteriaceae were detected in 429 (73.5%) out of the total 584 samples examined. Some samples yielded more than one ESBL-producing bacterial species. The ESBL-producing bacteria originated from humans (35/42; 83.3%), dogs (340/447; 76.1%), cats (5/13, 38.6%) and environmental samples (49/82; 57.8%) in Ibadan (180/239; 75.3%) and Lagos (249/345; 72.2%). ESBL-producing Enterobacteriaceae identified included *Escherichia coli* (n=279), *Klebsiella* spp (n=124), *Enterobacter* spp (n=6), *Proteus* spp (n=2), *Citrobacter* spp (n=3). In addition, *Pseudomonas* spp (n=15) were also detected among the ESBL-producing isolates. All tested isolates were resistant to ampicillin, ceftazidime and cefotaxime. There was 42.8% resistance to amikacin, 6.8% to cefoxitin, 31.2% to chloramphenicol, 93.5% to ciprofloxacin, 9.3% to ertapenem, 48.2% to gentamicin, 56.9% to kanamycin, 78.4% to nalidixic acid, 90.6% to streptomycin, 97.8% to compound sulfonamide, 89.5% to sulfamethoxazole/trimethoprim, 84.1% to tetracycline and 82.8% to trimethoprim. All the ten ESBL-producing *E. coli* from the same veterinary clinic examined by WGS possessed the *bla*CTX-M-15 ESBL gene. Moreover, four out of the ten isolates showed very close genetic relatedness. Four isolates originated from dogs (n=2) and humans (n=2). These four isolates all belonged to phylogroup B1, ST 730, serotype O91:H23 and possessed IncY *bla*CTX-M-15-carrying plasmids.

**Conclusions:** ESBL-producing Enterobacteriaceae are widely distributed among pets and in-contact humans in the study area. Findings from this study suggest possible nosocomial exchange of multidrug-resistant ESBL-producing *E. coli* between pets and humans in veterinary hospital settings.

## OP-017

# Detection of *bla*$_{\text{OXA-244}}$-positive *E. coli* in farm animals in the Netherlands

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**Background and objectives:** The worldwide increase in resistance to carbapenem antibiotics is considered a threat to public health [1]. To minimize the risk of an animal reservoir of carbapenemase producing Enterobacterales (CPE), the use of carbapenem antibiotics in animals is prohibited in the EU. Until 2024, CPE have not been found in farm animals or in nationally produced food within the AMR monitoring in the Netherlands [2]. In this study, we describe two positive CPE findings in samples from Dutch farm animals in 2024.

**Methods:** Within the framework of the AMR monitoring in animals and food, caecal samples from broilers, slaughter pigs and veal calves as well as faecal samples from dairy cattle were screened for the presence of CPE according to the most recent EURL-AR protocol. Since 2012, these samples are also screened for CPE using selective enrichment followed by multiplex PCR (targeting NDM, KPC, VIM, IMP, OXA-48, IMI and FRI/FLC) and subsequent culturing of PCR-positive samples. Antimicrobial susceptibility testing of CPE-suspected isolated was performed with broth microdilution in commercially available antibiotic panels (Sensititre ®, Thermo Scientific). Whole Genome Sequencing (WGS) was performed on Illumina MiSeq and Oxford Nanopore Technologies MinION, followed by hybrid assembly and sequence analysis.

**Results:** In 2024, CPE was detected in two caecal samples from different farms using the alternative PCR screenings method. The first positive sample originated from a flock of broiler chickens and the second from an individual fattening pig. In both samples, *E. coli* ST58 was identified with identical chromosomally located *bla*OXA-244 genes embedded between two IS-1 elements. Susceptibility testing of both *E. coli* isolates demonstrated low-level resistance to all carbapenem antibiotics tested.

Core-genome MLST analysis revealed genetical relatedness indicating a common source. Both samples were tested negative using the EUR-AR protocol. One *E. coli* showed poor growth on the selective media (CHROMID®, BioMerieux) and the other *E. coli* did not grow on these plates.

**Conclusions:** To the best of our knowledge, this is the first description of CPE in Dutch farm animals and the first description of *bla*OXA-244 in *E. coli* ST58. Our study demonstrates the importance of using a laboratory method which is capable of detecting CPE exhibiting low-level resistance to carbapenems like *bla*OXA-244.

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## OP-018

# Coexisting IncF plasmid allele variants impair *in silico* pMLST typing in epidemiologic surveillance

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**Background and objectives:** Plasmids of incompatibility group F (IncF) are plasmids that usually carry more than one replicon for the DNA replication initiation. The replicon sequence type (RST) is a significant parameter for determining the association of IncF plasmids with certain bacterial species and for understanding their evolution and epidemiology. During the analysis of IncF plasmid content in a collection of *Escherichia coli* ST131, inconsistent results were observed in the allele variants that define the RST. The study aimed to assess inconsistencies in IncF sequence typing and identify affected plasmid lineages by analysing an extensive collection of *E. coli* and other *Enterobacteriaceae* genomes.

**Methods:** In-house collection of ST131 from human, animal and environmental sources, public collection of genomes of various *E. coli* STs, and non-ST131 *E. coli* and *Enterobacteriaceae* in-house isolates (n=72469) were included. Representative ST131 isolates were long-read sequenced to confirm the alleles reported by pMLST and to identify their location in the genome. All sequences were subjected to IncF plasmid typing by pMLST analysis using three different versions of the pMLST tool.

**Results:** Inconsistent reports of IncF RST profiles were observed during repeated pMLST analysis with the RST not corresponding with the identified allele variants. Even though more than one replicon

allele variant was detected, this was not considered in the resulting RST profile. The occurrence of this phenomenon was relatively high with the multiple allele variants omitted in the RSTs with 8.01% frequency. Biologically, three different situations were observed: multiple allele variants on a single plasmid (F31/F36:A4:B1), multiple allele variants on two different plasmids (F1/F4:A2:B- being F1:A2:B- and F4:A-:B-), and overlapping allele variants (F18/C4:A-B1).

**Conclusions:** This study identified discrepancies between results reported by all versions of the *in silico* pMLST tools compared to the biological situation regarding multiple plasmid IncF allele variants. The pMLST tool performed incorrectly when designating ST and IncF alleles, particularly when multiple contigs were considered. Since plasmid STs are often linked to certain bacterial clones, improper pMLST report might lead to the omission of epidemiologically relevant associations.

## OP-019

# Genomic surveillance of ESBL/AmpC-producing *Escherichia coli* from livestock and comparative analysis of isolates from healthy and diseased cattle

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**Background and objectives:** Multidrug-resistant (MDR) *Escherichia coli* (ESBL/AmpC) represent a significant public health concern and due to their zoonotic potential are key targets for surveillance within a One Health framework. This study aimed to monitor their occurrence in healthy livestock in northern Spain, determine their resistance profile and characterise their whole genome. Furthermore, the genomes of isolates recovered from healthy cattle were compared with those of clinical isolates from diseased cattle.

**Methods:** In 2020-2023, samples were collected from 445 healthy animals at slaughterhouses in the Basque Country (northern Spain) as part of an AMR surveillance project. Rectal faeces (cattle, sheep, and swine) and caeca (chickens) were pooled in 89 composite samples (51 cattle, 8 sheep, 15 chickens, and 15 pigs). ESBL/AmpC-producing *E. coli* were isolated and subjected to antimicrobial susceptibility testing (MIC). Isolates representing diverse MDR phenotypic profiles (n=37) were sequenced with Oxford Nanopore Technologies (ONT), along with 26 previously characterised *E. coli* (ESBL/AmpC) isolates from bovine clinical cases. Genomic analyses included phylogroup determination, MLST, identification of antimicrobial genetic determinants of resistance (GDRs), plasmid replicon typing, pangenome, and core-genome phylogenetic analysis.

**Results:** ESBL/AmpC-producing *E. coli* were isolated from 47.2% of pools of samples from healthy animals, with pigs showing the highest proportion of positive pools**.** Seven phylogroups (A, B1, C, D, E, G, and clade I) were identified. B1 was the only phylogroup detected in all hosts. Chickens harboured three uncommon phylogroups (E, G and clade I). In bovine, phylogroups A, B1, and C were found in healthy and diseased cattle, but D and G were restricted to clinical isolates. Most of the 36 sequence types (STs) identified were unique and no STs were shared among all host species. Four STs (ST-10, ST-58, ST-88, and ST-744) were detected in healthy and diseased cattle. Resistance profiles varied by host, with bovine isolates showing the highest resistance rates, particularly to aminoglycosides, folate pathway antagonists, and tetracyclines. Bovine isolates also carried the highest number of GDRs, with more antimicrobial classes per isolate (5.5) than pigs (4.0), sheep (3.0), and chickens (2.0). GDR profiles were not associated with any particular host, phylogroup, ST, or health status as they were scattered across different hosts and phylogenetic groups with no clear clustering. GDR diversity was larger in cattle, followed by pigs, chickens and sheep, and in clinical isolates compared to isolates from healthy cattle. Most ARGs (71.9%; 455/633) were plasmids-borne, with a strong association between plasmid replicon type and the ARG profile. Of 227 detected plasmids, 40.5% carried ARGs, with ARG-rich plasmids (≥10 ARGs) being mostly found in diseased bovine isolates. The pangenome analysis identified a total of 29,337 genes, mostly made up of

accessory genes due to the great genomic diversity of the isolates. Core-SNP phylogeny correlated with phylogroup and ST but not with resistance profiles or health status.

**Conclusions:** This study highlights the high genomic diversity of MDR ESBL/AmpC-producing *E. coli* circulating in livestock in northern Spain. The higher genomic and resistance diversity observed in bovine isolates compared to chickens and pigs might reflect a more intensive farming practices and higher antimicrobial use as the poultry and swine isolates originated from free-range farming systems with minimal or no antibiotic use. The presence of common clonal lineages and plasmids in healthy and diseased cattle suggest active strain circulation. The predominance of plasmid-encoded ARGs is particularly concerning given their dissemination potential. These findings emphasize the need for continuous, integrated genomic surveillance in healthy reservoirs and clinical settings to monitor AMR emergence and spread.

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**OP-020**

## Dynamics of carbapenemase genes and producing-bacteria in groundwater, urban river and wastewaters to argue for an environmental cycle of antimicrobial resistance in a high-income metropole

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**Background and objectives:** Antimicrobial resistance (AMR) is a growing threat to public health, because it can compromise the treatment of certain severe infections, particularly those caused by Gram-negative bacilli such as *Enterobacteriaceae*, as well as invasive medical procedures and surgeries. Carbapenemase-producing bacteria (CPB) are part of the most concerning pathogen, by the production of carbapenemases, which are enzymes hydrolysing antibiotics of the carbapenems" family. These molecules are used as a last resort, to treat severe infections [1]. Several studies have highlighted the role of hydric environment in AMR emergence, spread and persistence. Hence, antimicrobial resistant bacteria and genes should be considered as major environmental contaminant [2, 3]. However, little is known about the dynamics of CPB, particularly those producing carbapenemase in water environments. This study investigates the distribution of carbapenemase genes (*bla*$_{KPC}$, *bla*$_{OXA-48}$, *bla*$_{NDM}$) and the presence of CPB in four contrasted water environments in Montpellier (France): wastewater (WW) from a university hospital and a municipal wastewater treatment plant (WWTP), and freshwater from the Lez underground spring and the Verdanson urban stream.

**Methods:** A total of 90 water samples were collected from the four water compartments. Total environmental DNA was extracted, to quantify carbapenemase *bla*$_{KPC}$, *bla*$_{OXA-48}$, and *bla*$_{NDM}$ as well as the IntI-1, IntI-2 and IntI-3 genes, using qPCR. These samples were also seeded onto chromogenic media supplemented with 0.125µg/mL and 2µg/mL meropenem, allowing to constitute a collection of carbapenem-resistant bacteria, which were all identified by MALDI-TOF-MS. 160 of these strains were then screened by specific PCR to detect the three carbapenemase genes of interest.

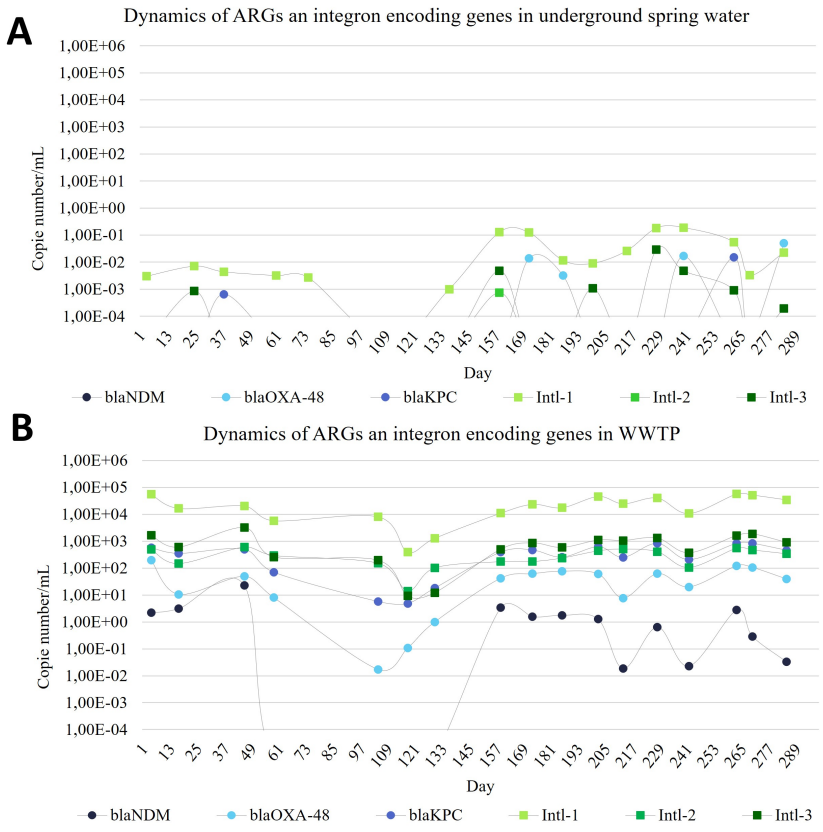
**Results:** The three carbepenemases genes were detected in almost all WW samples, with a maximal concentration of 4.47E+04 copies/mL in hospital WW, and 8.40E+02 in WWTP samples (Fig. B). However, *bla*$_{NDM}$ exhibited greater variability over time than *bla*$_{KPC}$ and *bla*$_{OXA-48}$. The inconsistent *bla*$_{NDM}$ signal over time suggests transient contamination rather than a stable environmental reservoir. Carbapenemase genes, except *bla*NDM, were also detected in several freshwater samples, in up to

63% of the urban stream and in 23% of the spring water at low concentrations, reaching a maximum of 1.45 copies/mL for the urban stream, and 3.67E-02 copies/mL for the spring (Fig. B). In WW and urban water, *bla*$_{OXA-48}$, *bla*$_{KPC}$ and the three integrons encoding genes follow the same temporal dynamics (Fig. A and B). Moreover, environmental factors such as precipitation and temperature had limited influence on genes load except for samples from WWTP that showed significant variations depending on temperature and seasonality ($p<0.05$), with a decrease of at least 1.00E+02 gene copies/mL for all the genes tested between December and February (Fig. B).

The culture-based approach allowed the isolation of 6 864 carbapenem-resistant bacteria, mainly belonging to *Pseudomonas, Acinetobacter, Citrobacter*, and *Enterobacter* genera. Among the 160 tested strains from the hospital WW, 36 (23%) carried at least one of the three carbapenemase genes of interest, *bla*$_{OXA-48}$ being the most frequently detected (16% of the strains). Furthermore, the majority of the carbapenemase-producing bacteria belong to environmental-associated genera *Aeromonas* (11/22 tested strains), *Shewanella* (8/15 tested strains), *Citrobacter* (7/14 tested strains), *Comamonas* (4/18 tested strains) and *Pseudomonas* (3/39 tested strains). Moreover, in the spring, 3/9 of the tested enterobacteria carried carbapenemase genes.

**Conclusions:** This study provides critical elements in favor of an established environmental cycle carbapenemases genes and carbapenemase-producing enterobacteria in a french metropole: i) carbapenemases genes and carbapenemase-producing enterobacteria detection in spring groundwater (*Klebsiella*, *Enterobacter*); ii) environmental-borne carbapenemase-producing bacteria in human WW (*Pseudomonas, Aeromonas, Shewanella, Comamonas*...). An environmental cycle increases the risk of spreading human pathogenic bacteria carrying carbapenemase genes. Thus, there is a need for a monitoring system of environmental antimicrobial resistance. Recent developments of wastewater based epidemiology is applicable for antimicrobial monitoring, but the complexity of the WW microbial communities, which is a hot-spot for gene transfers, could blur the epidemiological signal. Monitoring antimicrobial resistance genes in fresh water could be a useful alternative to follow the epidemiology of emerging resistance with environmental cycle.

**Figure**



**OP-021**

## NGS-based molecular typing of veterinary *Escherichia coli* strains in Germany between 2004 - 2022 from the German national antibiotic Resistance Monitoring (GE*RM*-Vet)

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**Background and objectives:** Livestock-associated *E. coli* are ubiquitous in food-producing animals. Most strains are commensals that seldom cause diseases. However, the spread of commensal and pathogenic *E. coli* harbouring antimicrobial resistance (AMR) determinants within and across animal and human populations presents a growing threat to global health. Among others, colistin resistance in multidrug-resistant (MDR) bacteria is a significant public health concern. The relevance of livestock for the transmission of pathogenic MDR bacteria across host populations remains unclear.

**Methods:** The Federal Office of Consumer Protection and Food Safety (BVL) collects pathogenic *E. coli* from diseased pigs, cattle, poultry and horses as well as from companion animals such as dogs and cats as part of the GE*RM*-Vet study. AMR was determined by the broth microdilution method according to CLSI guidelines. Illumina MiSeq-sequencing and bioinformatic analyses were performed to identify and characterize 1140 isolates collected between 2004 and 2022. A cgMLST minimum-spanning tree was built to illustrate clonal relationships.

**Results:** A total of 171 different sequence types were detected. The NGS analysis showed mostly a distinctive association between sequence type and host species. For instance, the most common sequence type was ST1 with 99.5% of the strains isolated from diseased pig, followed by ST10 strains with 64% from pig, 32% from cattle, 3% from horse and 1% from poultry. Among isolates with an elevated colistin minimum inhibitor concentration (≥2mg/L), an increase of mobile colistin resistance (mcr) genes over chromosomal mutations was observed over time. The earliest detection of mcr genes was *mcr*-5 in 2004, which was superseded by *mcr*-1 as the dominant variant in 2009. For 9.3% of the isolates examined with an elevated colistin MIC value, no resistance mechanism could be detected.

**Conclusions:** Our retrospective analyses show both growing clusters of dominant sequence types and a consistently high proportion of rare sequence types. Isolates with unknown resistance mechanism should be investigated further. From a One Health perspective, whole genome sequencing makes an important contribution to epidemiological surveillance in the veterinary sector.

**OP-022**

## Whole-Genome Sequencing Analysis of Non-Typhoidal *Salmonella* Isolated from Animals at Slaughter in the Philippines Provides Insights into Circulating Serovars and Antimicrobial Resistance

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**Background and objectives:** Non-typhoidal *Salmonella* (NTS) demonstrates the One Health paradigm, as reducing human infections will require the control of NTS in animals and limitation of transmission from the environment. In the Philippines, chicken and pig meat have been identified as the dominant food vehicles for transmission to humans. Due to frequent antibiotic exposure of animals, meat products are recognised as risks in disseminating multidrug resistant (MDR) strains. The application of Whole Genome Sequencing (WGS) into routine surveillance of foodborne pathogens has not yet been integrated in the Philippines. This study aimed to use WGS in determining the serovars and antimicrobial resistance (AMR) genotypes of NTS isolated from slaughtered animals.

**Methods:** Ninety-two NTS isolates collected in 2022 and 2023 from slaughtered chickens (60), pigs (28), cattle (2), carabao (1) and duck (1) from 12 provinces in the Philippines were analysed. Isolation and identification were carried according to ISO6579-1:2017. Minimum Inhibitory Concentration for 15 antibiotics representing nine antibiotic classes was determined by broth microdilution and interpreted using the EUCAST ECOFFs. WGS was performed using Illumina HiSeq platform. Short-read Illumina sequences were analysed using NCBI AMR Finder Plus and APHA SeqFinder to determine the presence of AMR genes. Genomic diversity was assessed by determining the Sequence Type and phylogenetic trees were constructed using core genome SNPs. Serovar determination was carried out using APHA"s ISO 17025:2017 accredited bioinformatics pipeline. Long-read sequencing was conducted on selected isolates (n=5) harbouring resistance to highest priority critically important antibiotics (HP-CIAs).

**Results:** Predominant serovars included Enteritidis (29.3%), Infantis (22.8%), Typhimurium (8.7%), and Kentucky (2.2%), serovars previously reported for human NTS infections in the Philippines. There was excellent concordance between resistance phenotype and genotype. Ciprofloxacin resistance was detected in 58 isolates, associated with *gyrA* mutations (n=51) or *qnrS* (n=7). Forty-four (47.8%) isolates were MDR; 26 representing four serovars were ESBL-producers, harbouring *bla*CTX-M-65 (n=20) or *bla*CTX-M-123 (n=6). Seventeen MDR Infantis isolates carried the IncFIB pESI-like plasmid containing *bla*CTX-M-65 and 7-12 other AMR genes. Potential clones (core genome SNP-dist <10) harbouring resistance to HP-CIAs were identified in this serovar. An MDR *S.* Kentucky (ST198) carried an IncX4 plasmid harbouring 8 AMR genes including *mcr-1*. AMR genes conferring resistance to HP-CIAs were carried on all plasmids examined, with homology to plasmids previously described in isolates from livestock and human.

**Conclusions:** The current findings have filled important evidence gaps by providing detailed description of the AMR resistance patterns, serovar identity, and genomic diversity of *Salmonella* from meat entering the food chain. MDR *Salmonella* are associated with more serious diseases in people and ciprofloxacin resistant *Salmonella* are classed as a WHO priority pathogen. These data therefore contribute to the One Health approach to tackling AMR and can be employed alongside human and environment data sets to assess risk and monitor interventions.

## OP-023

# Monitoring of antimicrobial susceptibility of *Streptococcus suis* in the Netherlands, 2020-2024

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**Background and objectives:** *Streptococcus suis* (SSU) is a frequently occurring, important pig pathogen and has serotype dependent zoonotic capacity. Access to representative, reliable antimicrobial susceptibility (AMS) testing data is a prerequisite for prudent use of antibiotics in treatment of SSU infections in pigs. The objective of this study was to analyse the *in-vitro* AMS of SSU from samples from diseased pigs in the Netherlands.

**Methods:** SSU isolates, almost 2500, originated from diagnostic submissions of pigs sent from Dutch pig farms to Royal GD, from January 2020 until December 2024. Minimal inhibitory concentrations

(MICs) of 11 antimicrobials were assessed by broth microdilution following CLSI recommendations. $MIC_{50}$ and $MIC_{90}$ values were determined and MICs were interpreted as susceptible, intermediate and resistant using CLSI veterinary breakpoints (when available).

**Results:** Emergence of resistance among SSU from diseased pigs appeared to be limited. Percentage of resistance to ampicillin, ceftiofur, enrofloxacin, florfenicol, penicillin, and trimethoprim/sulfamethoxazole was low, to clindamycin medium, and to tetracycline high. Cross-resistance between penicillin and ampicillin appeared to be incomplete. For several antimicrobials, an effect of age category on MIC values and percentages of resistance was found.

**Conclusions:** Previous research has shown that the number of SSU isolates is well representative considering the number of pigs (and number of farms) per province they originate from. Therefore, this passive monitoring is considered to provide a reliable and representative picture of the AMS of SSU isolates in the Netherlands. Interpretation of MICs of (clinically relevant) antimicrobials tested for treatment of SSU infection is strongly hampered by the lack of clinical breakpoints that are animal species- and body-site-specific. Therefore, and to conduct a clinically reliable monitoring of AMS of veterinary pathogens, more species- and body-site-specific veterinary breakpoints are urgently needed. Additionally, to further refine antibiotic treatment in pigs, it should be considered to what extent possible effects of age or preceding antibiotic treatments affect treatment choice.

## OP-024

# The genome sequence of the hedgehog-associated *mecC*-MRSA CC599

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**Background and objectives:** After discovery of *mecC*, an alternative gene conferring beta-lactam resistance to *Staphylococcus aureus*, epidemiological studies indicated a zoonotic background of *mecC*-MRSA. These strains are associated with hedgehogs (*Erinaceus europaeus*) where they evolved to adapt to selective pressure exerted by penicillin-producing dermatophytes (*Trichophyton erinacei*). CC599 is one of these *mecC*-MRSA lineages, and aim of the study was to characterise it further.

**Methods:** A *mecC*-MRSA isolate originated from a nasopharyngeal swab of a road-killed hedgehog from Jena, Thuringia. It was identified and characterised by DNA-microarray and then subjected to Nanopore sequencing. Additional isolates were cultured from swabs or homogenised ticks obtained from live hedgehogs. Clinical isolates originated from routine diagnostics or from screening of hospital patients. All isolates were typed using DNA-microarrays.

**Results:** The isolate had the sequence type ST599, *spa* type t5930 and carried an SCC*mec* XI element identical to previously sequenced elements form other *mecC*-MRSA lineages (CC130, CC425; GenBank CP155062.1 and FR821779.1). This also included the presence of a specific penicillinase gene and an arsenic resistance operon. It carried two pathogenicity islands, one of them harbouring genes for enterotoxins C and L, and for the toxic shock syndrome toxin. Other virulence

factors included enterotoxin genes *seli2=sel26* and *selu2=sel27* as well as leukocidin genes *lukD/E.* There was one prophage, integrated at an unusual site between *glpD* and *miaA* (cgMLST SAUR1305 and -1307).

In hedgehogs, this strain is not uncommon. Two out of three MRSA isolates found among additional 59 hedgehogs sampled in 2024 in and around Jena belonged to it (while the third one was CC130-MRSA-XI). In contrast, CC599-MRSA-XI is rare among humans. Microarray-based typing of ca. 1800 clinical MRSA isolates collected during 20 years at the Dresden University Hospital yielded not a single isolate. Among 3000 clinical MRSA collected during ten years at Regensburg University Hospital, five belonged to this strain.

**Conclusions:** CC599-MRSA-XI is a "hedgehog-associated" MRSA strain carrying *mecC* on the same SCC*mec* element as other *mecC*-MRSA. It carries virulence factors that are also relevant to humans, and there might be some zoonotic potential.

## OP-025

# Staphylococcus aureus carriage in horses in Norway

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**Background and objectives:** *Staphylococcus aureus* is a versatile bacterial species, which have adapted to diverse set of hosts. This includes horses, where it"s a well-recognized opportunistic pathogen primarily associated to skin and soft tissue infections. Research on horses has however mainly focused on methicillin-resistant *S. aureus* (MRSA) and carriage and infection by MRSA in horses are primary due to a horse-adapted linage of the Livestock-associated (LA)-MRSA clonal complex (CC)398. Studies on *S. aureus* as a commensal in horses remain limited, with carriage rates varying from 0 to up to 40 %. While some *S. aureus* from horses likely originate from humans there also appears to be horse-adapted strains belonging to CC1, CC8 and CC398. The main objective of the study was to determine the carriage of both MRSA and methicillin-sensitive *S. aureus* (MSSA). It also aimed to evaluate antibiotic susceptibility of the isolates, and to perform genotypic characterization to identify genetic linages, potential relatedness, and identification of genetic markers associated to antibiotic resistance, virulence and host-adaption.

**Methods:** Veterinarians in Norway was encouraged to submit samples from horses collected from the muzzle using swabs. Information on age, municipality, breed, use, if it has been abroad, clinical symptoms at sampling and treatment with antibiotics in the last three months were also recorded. Samples should be sent to the Norwegian Veterinary Institute (NVI) the same day of sampling, but could be stored for a max of two days in a refrigerator. On arrival to the NVI, swabs were incubated in Mueller Hinton broth supplemented with 6.5% NaCl, and from overnight broths, 10 µl was streaked respectively on bovine blood agar, Mannitol Salt Agar, and Brilliance™ MRSA 2 Agar (Thermo Fisher Scientific), which were incubated according to instructions by the manufacturer. Suspected *S. aureus* isolates were re-streaked on blood agar and the species was identified by Maldi-TOF (Bruker). Confirmed *S. aureus* were subjected to in-house genome sequencing using Illumina-based technologies and subsequent bioinformatics analyses. Minimal inhibitory concentration (MIC) to antibiotics were determined using SensititreTM EUST2 (Thermo Fisher Scientific).

**Results:** Between March - December 2024, 251 samples were submitted, to NVI, with no samples positive for MRSA. MSSA were found in 18% of the horses (n = 45). Additionally, other *Staphylococcus* spp. were detected, with *S. delphini*, a member of the coagulase-positive *S. intermedius* complex, being the most common, found from 12% (n = 31) of horses. The *S. delphini* isolates were not characterized further. The genome sequencing and MIC-determination is currently ongoing, but the isolates that so far have been sequenced belongs to clonal complex (CC)1. All analysis and results are expected to be completed in May 2025.

**Conclusions:** The occurrence of MSSA among horses in Norway appears to be slightly higher than previously reported from Europe. Notably, no MSRA were detected in the Norwegian horse population. Genotypic characterization and antibiotic susceptibility testing of all MSSA isolates are currently ongoing

## OP-026

# China Antimicrobial Resistance Surveillance Network for Pets (CARPet), 2018 to 2022

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**Background and objectives:** Monitoring antimicrobial resistance (AMR) is essential to guide treatment and stewardship policies. While China has implemented AMR surveillance systems in human and food animals, pet-associated AMR remains understudied. Consequently, China Agricultural University launched China Antimicrobial Resistance Surveillance Network for Pets (CARPet) in 2021 to monitor the resistance profiles of clinical bacteria from pets.

**Methods:** China Agricultural University Veterinary Teaching Hospital obtained clinical samples, isolated, identified, preserved bacteria from samples of pet hospitals across China. Antimicrobial susceptibility testing of pet-derived isolates was performed using the broth microdilution method with custom-made broth microdilution panels (Thermo Fisher Scientific) according to CLSI documents VET 01S. Results were interpreted according to the breakpoints in CLSI VET01S and M100, and data was analyzed with WHONET software version 2022.

**Results:** Between 2018 and 2022, we recovered and tested the antimicrobial susceptibility of 5,637 isolates from dogs and cats across 25 Chinese provinces. The most predominant bacterial species were *Escherichia coli* (19.0%), *Staphylococcus pseudintermedius* (17.8%), *Pseudomonas aeruginosa* (5.7%), *Enterococcus faecium* (5.3%) and *Enterococcus faecalis* (4.9%). Enterobacterales showed highly susceptible to tigecycline, meropenem, colistin and amikacin (70.3%-100.0%), but moderate resistance to ampicillin, ceftriaxone, doxycycline, florfenicol, levofloxacin, enrofloxacin, and trimethoprim-sulfamethoxazole (29.3%-57.8%). Approximately 65.0% of *Acinetobacter* spp. were resistant to florfenicol, with relatively low resistance to another 11 antimicrobial agents (1.0%-25.2%). *Pseudomonas* spp. were high susceptibility to colistin (93.5%) and meropenem (88.9%). Coagulase-positive *Staphylococcus* spp. had higher resistance rates to most antimicrobial agents than coagulase-negative *Staphylococcus* isolates. However, over 90.0% of *Staphylococcus* spp. were susceptible to linezolid, daptomycin and rifampin, with no vancomycin-resistant isolates detected. *E. faecium* isolates (34.6%-97.3%) demonstrated higher resistance rates to enrofloxacin, florfenicol, rifampin, doxycycline and azithromycin than *E. faecalis* isolates (18.2%-86.4%).

**Conclusions:** Collectively, resistance of clinical isolates to commonly used antimicrobial agents is prevalent in pets, suggesting that it is urgent to strengthen the monitoring of AMR in pets. By timely and effectively collecting, analyzing, and reporting antimicrobial resistance dynamics in pets, the CARPet network will become a powerful platform to provide scientific guidance for both pet medical care and public health.

## *Session 4 - Understanding the connection of antimicrobial resistance between Animals, Humans and/or the Environment*

**OP-027**

# Temporal association between antimicrobial usage in livestock and antimicrobial resistance in human *Salmonella*, *Campylobacter* and *E. coli* infections in the Netherlands

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**Background and objectives:** Since 2009, antimicrobial usage (AMU) in livestock has decreased substantially in the Netherlands. The extent to which this decrease has affected levels of antimicrobial resistance (AMR) in human infections remains unclear. In this study we assessed the temporal association between AMU in livestock and AMR in human zoonotic infections caused by non-typhoidal *Salmonella* [*S.* Enteritidis and *S.* Typhimurium (including its monophasic variant)], *Campylobacter* (*C. jejuni* and *C. coli*), and in urinary tract infections (UTIs) caused by ubiquitous *Escherichia coli*.

**Methods:** An ecological registry-based study was conducted, using data from national surveillance programmes on AMR and AMU in livestock and humans between 2004-2020 (exact range varied per micro-organism). Associations were studied per micro-organism/serotype, antimicrobial class and livestock species, using logistic regression and correlation analysis.

**Results:** For *S.* Typhimurium, mainly positive associations were found between AMU in livestock and AMR in human infections. Significant positive associations were observed between AMU and AMR in broilers/pigs. No significant correlations were found between AMR in broiler/pig and human isolates. Most associations for S. Enteritidis were non-significant. For *Campylobacter*, AMU in livestock was negatively associated with AMR among human infections, while positive correlations were found between AMR in broiler and human isolates. For *E. coli*, both positive and negative associations between livestock AMU and human AMR were observed. Generally, stronger associations were found between AMU and AMR within the same population (human/livestock).

**Conclusions:** Overall, the associations between AMU in livestock and AMR in human zoonotic infections are not consistent. The negative associations observed for *Campylobacter* emphasize that reducing AMU in livestock alone may not be enough to tackle increasing AMR in humans. Furthermore, the marginal association between livestock AMU and human AMR in *E. coli* UTIs provides further evidence that the zoonotic spread of *E. coli* causing UTIs is relatively limited.

**OP-028**

# *Escherichia coli* resistance genes spread between humans, animals and environment within households, via plasmid-mediated horizontal transfer, in Battambang province, Cambodia

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**Background and objectives:** Intensive antibiotics use in human and veterinary medicine has spurred the rise of increasingly resistant strains. Models predict that Antimicrobial Resistance (AMR) could cause 10 million annual deaths by 2050. Cambodia faces a high and rising incidence of Extended Spectrum Betalactamases-producing (ESBL) *Enterobacteriaceae* and Carbapenemase-producing (CP) *Enterobacteriaceae*, which are classified as critical bacterial priority pathogen by the WHO. Health policies acknowledge the urgent necessity to understand the biological determinants of AMR emergence and spread between humans, animals and environmental compartments. Accordingly, our project explores *Escherichia coli* populations and their resistomes (all their Antibiotic Resistance Genes – ARGs) at compartments" interfaces, shedding light on AMR circulation in Battambang Province, Cambodia.

**Methods:** Patients with ESBL or CP *E. coli* infections at Battambang Hospital were included. Environment (water, soil, food) and domestic animals and rodents (rectal swabs) were sampled in their households. Bacterial culture, mass spectrometry and antibiograms allowed strain isolation, species identification and resistance phenotype determination respectively. Each strain"s DNA was extracted and sequenced on an Illumina instrument. Sequences were submitted to Baargin [1], followed by phylogenetic and statistical analyses of its output files.

**Results:** We studied 189 *E. coli* strains isolated in 21 households. 83.6% of these strains were ESBLE and 1.6% were CPE, with ARGs responsible for the resistant phenotypes detected on plasmid sequences in 100% of the resistant strains. Multi Locus Sequence Typing detected 89 different STs including 11 ST131 and 9 ST1193 in patient, animal and environment samples. Phylogenetic analysis showed no clustering per household nor phenotype (Figure). The Principal Component Analysis (PCA) of the resistance profiles showed only a clustering per sample type, with patient"s E. coli profiles separated from animals and environment"s E. coli profiles. A distance analysis revealed that the patient strains" resistome was significantly similar to environmental and animal strains" resistomes within a household, whereas food strains" resistomes were significantly different.

**Conclusions:** This study highlights the circulation of very diverse *E. coli* strains in Battambang including the high-risk multi-resistant clones ST131 and ST1193, detected in patients, animals and in their environment.

The absence of spatial clustering of resistomes and STs suggests a broad dissemination of resistant strains at the provincial level. The difference of patients" resistomes from animal and environment resistomes observed in the PCA analysis can be attributed to the fact that patient strains originate from bacterial infections with distinct biological characteristics, in terms of virulence and pathogenicity.

The intra-households versus inter-household resistome comparison using distance analysis showed a significant similarity of patient, animal and environment resistomes within households, suggesting a privileged transmission of ARGs between these compartments within households. The absence of strain clustering by household, the significant similarity of AMR profiles within households and the plasmid carriage of the genes conferring the ESBL and CPE phenotypes suggest that this ARGs circulation is plasmid-mediated.

Overall, this study suggests different patterns of circulation between bacterial strains and ARGs, given evidence of independent transmission of ARGs, most likely mediated by plasmids. A global exploration of plasmids is currently underway, in order to further characterize the mechanisms of this circulation. Such data is essential to assess the risks for both humans and animals, and for implementing preventive measures aimed at limiting the emergence and spread of AMR in Cambodia, where antibiotic misusage is widespread and poorly regulated across healthcare facilities, communities, and agricultural practices.

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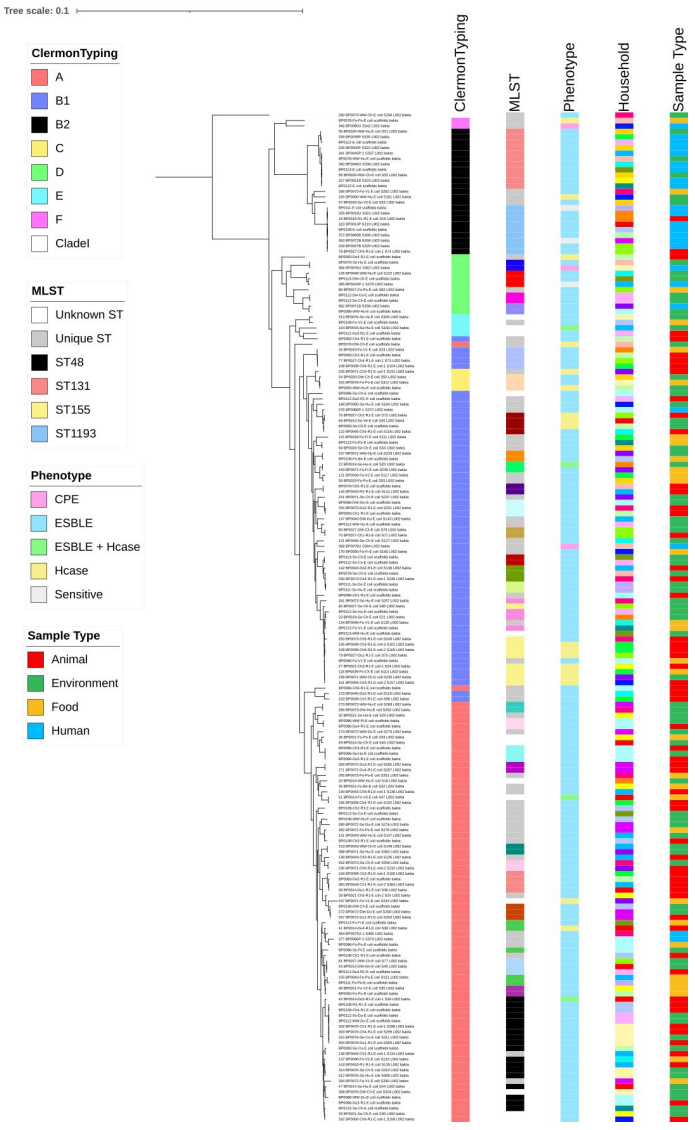
## Figure



**OP-029**

# Human-associated phylogroups are common among extended-spectrum β-Lactamase-producing *Escherichia coli* isolates from Viennese dogs

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**Background and objectives:** Companion animals, such as dogs, have been found to carry antibiotic-resistant bacteria, including the highly clinically relevant extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-E) [1]. Considering the unique role that dogs occupy in the zoonotic transmission network due to the frequent and high-intensity contacts with their owners, other animals and the environment, it is important to understand the prevalence of ESBL-E among dogs, the population structure and phylogenetic relationships of these isolates as well as the genetic determinants of resistance to other clinically relevant antibiotics and virulence factors.

**Methods:** Fecal samples of 88 dogs attending the small animal university clinic were plated on selective agar (MacConkey agar supplemented with 2 µg/ml cefotaxime), bacterial isolates were identified to a species level by MALDI-ToF, tested for phenotypic resistance by disk diffusion and Vitek 2, whole-genome sequenced by Illumina and bioinformatically analysed.

**Results:** Thirteen ESBL-E were isolated in 88 dogs, reaching the prevalence of 14.8 % (95%CI: [8.1 - 23.9]). Phylogenetic analyses revealed a highly diverse population structure, with over half of the isolates belonging to human-associated intestinal (phylogroups C and A) and extraintestinal (phylogroups D, E, B2) phylogroups, while four isolates belonged to the environment-associated phylogroup B1. Among the 13 isolates, 12 different sequence types (STs) were identified, including high-risk human-associated international clones of ST38, ST131 and ST141. Core-genome Neighbour-Joining trees showed that the isolates from ST38 and ST141 clustered most closely with human clinical isolates, while the isolate from ST131 clustered with isolates from both humans and pets. The majority of the isolates could be classified genotypically as multi-drug resistant and all isolates encoded various virulence-associated genes, including exotoxins in the pandemic ST131 and emerging ST141 isolates.

**Conclusions:** A relatively high number of dogs in Vienna are colonised by ESBL-E, the majority of which belongs to the human-associated phylogroups including the pandemic lineages ST131, ST141 and ST38. This underlies the need for adopting the One Health strategy in epidemiological surveillance of ESBL-E at the human-dog interphase.

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**OP-030**

# From Farm to Fork: Transmission Dynamics of NDM-CRE in Broiler Production Chain and Implications for Public Health

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**Background and objectives:** Carbapenem-resistant *Enterobacterales* (CRE) represent a critical global health threat with increasing mortality rates globally [1]. Despite carbapenem prohibition in food animals, New Delhi metallo-β-lactamase producing CRE (NDM-CRE) of animal sources continue rising [2]. This study aimed to systematically investigate the prevalence, sources, and transmission dynamics of NDM-CRE throughout the broiler production chain, highlighting public health concerns.

**Methods:** A longitudinal study was conducted across three broiler farms, including their upstream hatcheries and breeder farms, and downstream abattoirs and food markets. In total, 7,372 samples were collected and selectively cultured on MacConkey agar plates containing 0.5 mg/L meropenem. A subset of 861 representative *bla*$_{NDM}$-positive isolates were subjected to whole-genome sequencing (WGS). Additionally, a nationwide survey of 115 poultry farms from 11 provinces in China was performed to evaluate the national prevalence of NDM-CRE.

**Results:** The overall prevalence of NDM-CRE was 43% in broiler farms, 10.8% in breeder farms and hatcheries, and 61.3% in abattoir and retail samples. Notably, NDM-CRE persistence in the vacant farm environment rapidly colonized chick intestines (30-60%) within six hours post-placement. Additionally, hatcheries are also identified as sources of NDM-CRE in broiler farms, with both chicks and transport equipments serving as vectors. WGS analysis revealed the clonal transmission of NDM-CRE within and across farms, batches, and production stages, impacting chickens, carcasses, and retail meat products, presenting the first conclusive evidence of antimicrobial resistance (AMR) transmission from farms to meat products. Furthermore, *bla*$_{NDM}$-encoding plasmids, predominant of IncHI2 and IncX3, transmitted to other *Enterobacterales* strains, forming a complex CRE populations throughout the production chain. The nationwide survey corroborated these findings, revealing widespread NDM-CRE contamination in broiler farms (93.5%), breeder farms (88.5%), hatcheries (56.7%), and layer farms (46.2%).

**Conclusions:** This study identifies hatcheries and contaminated farm environments as primary sources of NDM-CRE in broiler farms, and provides direct evidence of their transmission from broiler farms to retail meat. These findings underscore the urgent need for targeted interventions at critical control points to mitigate the spread of AMR and reduce potential public health risks posed by contaminated food products.

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**OP-031**

# Direct-fed microbial administration to reduce antimicrobial resistant enterococci in beef cattle and the feedyard environment to promote One Health

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**Background and objectives:** Humans can be exposed to antimicrobial resistant (AMR) bacteria from food animals through direct contact, contaminated meat products, feedyard runoff, manure used as fertilizer, and airborne particulate matter. Multiple researchers have shown that airborne particulate matter originating from food animal production systems can transfer AMR organisms and other resistance determinants to surrounding areas; however, further work is needed to characterize intervention strategies that influence the abundance of AMR enterococci in the feedyard environment or the airborne particulate matter that arises therein. Direct-fed microbials (DFM) have been suggested as a strategy to reduce AMR within and around food animal production systems. Previously, our research group revealed that oral administration of a DFM was associated with a progressive shift among *E. faecium* isolates in cattle feces, where the probiotic sequence type (ST) 296 increased over time concomitant with a decrease in the more pathogenic ST240, which commonly carries both *ermB* and *tet*(M) resistance genes. These findings suggest that this DFM may competitively exclude more pathogenic STs in cattle feces; however further research is needed to determine DFM viability in the environmental manure pack and airborne particulate matter. Moreover, information regarding population dynamics among the DFM strain and wild type enterococci within the feedyard environment during the finishing period is limited. The objective of this study was to utilize environmental pen level samples to investigate the effects of tylosin and an *Enterococcus faecium/Saccharomyces cerevisiae* DFM on the abundance and AMR among enterococci in the cattle feedyard environment and airborne particulate matter.

**Methods:** A longitudinal trial in finisher beef cattle with pen-level treatment groups that included 1) DFM, 2) tylosin, 3) DFM/tylosin, and 4) untreated control was conducted over 119 days in Texas. Tylosin was withdrawn on day 84 for subsequent washout, while DFM administration continued to the end of the study on day 119. Environmental manure pack samples were collected on days 0, 84 and 119 of the study; and day 84 samples were further processed to model the desiccation of manure pack into particulate matter that naturally occurs over time in the feedyard environment. *Enterococcus* was quantified in triplicate with one presumptive *E. faecium* isolated per plate. MALDI-TOF confirmed *E. faecium* isolates underwent phenotypic antimicrobial susceptibility testing, a subset of which were further characterized using whole genome sequencing (WGS) (n=160) to define phylogenetic relatedness, AMR genes, virulence factors, plasmids, and other determinants of AMR.

**Results:** *Enterococcus* demonstrated increased resistance to tetracycline and erythromycin from day 0 to 84 of the study, followed by a subsequent decrease in resistance to day 119. Phenotypic resistance to erythromycin and tetracycline was detected in 72% and 87% of *E. faecium* isolates, respectively. WGS revealed a diverse set of sequence types and genotypic AMR profiles. Resistance genes were detected in the majority of isolates that exhibited phenotypic resistance. Most interestingly, two *E. faecium* isolates clonal to the DFM strain (ST296) were isolated from artificially produced environmental samples; both isolates were obtained from DFM only treatment pens and did not show evidence of the acquisition of AMR genes, plasmids, or other AMR determinants.

**Conclusions:** DFMs may reduce the risk of AMR in the feedyard environment and airborne particulate matter through faecal-environmental-oral cycling more effectively than previously understood.

Understanding the dynamics that facilitate ongoing faecal-environmental-oral cycling of DFM strains in feedyard production systems may reveal strategies to mitigate the risk of AMR bacteria emissions via bio aerosolized particulate matter from the feedyard environment to surrounding communities.

**OP-032**

# Multi-pathway Quantitative Microbial Risk Assessment Model for Evaluating Human Exposure to ESBL-Producing *E. coli* from Broiler Production

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**Background and objectives:** Broiler manure is an effective soil fertilizer but also a potential source of antimicrobial-resistant bacteria, including ESBL-producing *Escherichia coli* (*E. coli*). This study, performed within the framework of the ENVIRE project, aimed to quantify ESBL *E. coli* concentrations in broiler manure, identify transmission routes from manure to the environment, estimate human exposure via different pathways, and evaluate the effectiveness of interventions to reduce the exposure.

**Methods:** An integrated quantitative microbial risk assessment model was developed, encompassing three modules: farm, environmental, and exposure. The farm module simulated ESBL *E. coli* transmission and excretion within a broiler flock. The environmental module assessed bacterial survival, decay, and transport following manure application, employing exponential decay models fitted to empirical data for the soil, and Mancini's equation for bacterial decay in water. It modeled soil contamination post application of several types of manure (fresh, composted, stocked, anaerobically digested at 30°C and 37°C, and short-term stored) and bacterial transport to river systems caused by runoff events. Human exposure was estimated through lettuce consumption and recreational water activities, incorporating biphasic decay and Monte Carlo simulations to account for variability and uncertainty.

**Results:** At 39 kg/m² stocking density, ESBL *E. coli* concentration reached 1.6 × 10^4 CFU/g manure (SD: 0.02 × 10^4) by day 36. Consuming 100g of unwashed lettuce led to an exposure of 0.003-0.975 CFU, depending on the planting waiting period post manure application. An adult swimming for an hour in a contaminated bathing site was exposed to 1.11^-9 - 1.44^-6 CFU/g, depending on the interval between manure application and exposure. Composting and stocking manure eliminated ESBL *E. coli* before application, while short-term storage achieved around 99% reduction. Anaerobic digestion effectiveness was temperature-dependent, with digestion at 37°C outperforming 30°C.

**Conclusions:** Effective manure management practices significantly mitigate environmental and human exposure to ESBL-producing *E. coli*. Ensuring sufficient waiting periods post-manure application drastically reduces contamination risks associated with fresh produce consumption and recreational water activities.

**OP-033**

# Determination of the resistome of hunted wildlife animals in two East German regions by using capture hybridization enrichment

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**Background and objectives:** Antimicrobial resistance genes can be spread through vertical and horizontal gene transfer between different One Health sectors. In this context, wildlife might act as vehicles for the transfer of AMR bacteria at the human-livestock-wildlife interface due to their relatively large home range. By assessing resistomes in fecal samples from hunted animals, we aimed to investigate the occurrence of AMR genes in different animal species as a basis for assessing possible transmission pathways.

**Methods:** The samples were collected as part of a standardized wild animal sampling approach, which has been shown to be representative for hunted game species and effective in a large number of samples for various food safety studies in Germany, allowing long-term monitoring of biological and chemical risks [Maaz et al. 2022].

Fecal swap samples were taken directly from the hunted animals in the field. Samples were enriched in 10ml buffered peptone water at 37°C for a max. of 24h. For each hunting event, the enrichment cultures from the same animal species were pooled (max. 5 samples per pool). DNA was extracted with Qiagen PowerFecalPro Kit using 1ml of the pooled enrichment culture. The sequencing library was prepared using Illumina DNA Prep, (M) Tagmentation (96 Samples) und Primer IDT® for Illumina Nextera DNA Unique Dual Indexes. These libraries were used for enrichment of antimicrobial resistance genes by bait-based hybridization capture enrichment (AMR-Cap-Antimicrobial Resistance Gene enrichment panel, myBait, Daicel arbor biosciences)[Beaudry et al. 2021]. The libraries of four samples were pooled per enrichment reaction and sequenced to a depth of 162.5 Mbp (1.1 mio reads) per sample. Resistance genes were determined by analyzing trimmed raw reads in ResFinder 4.0 with thresholds of 0.9 for identity and coverage.

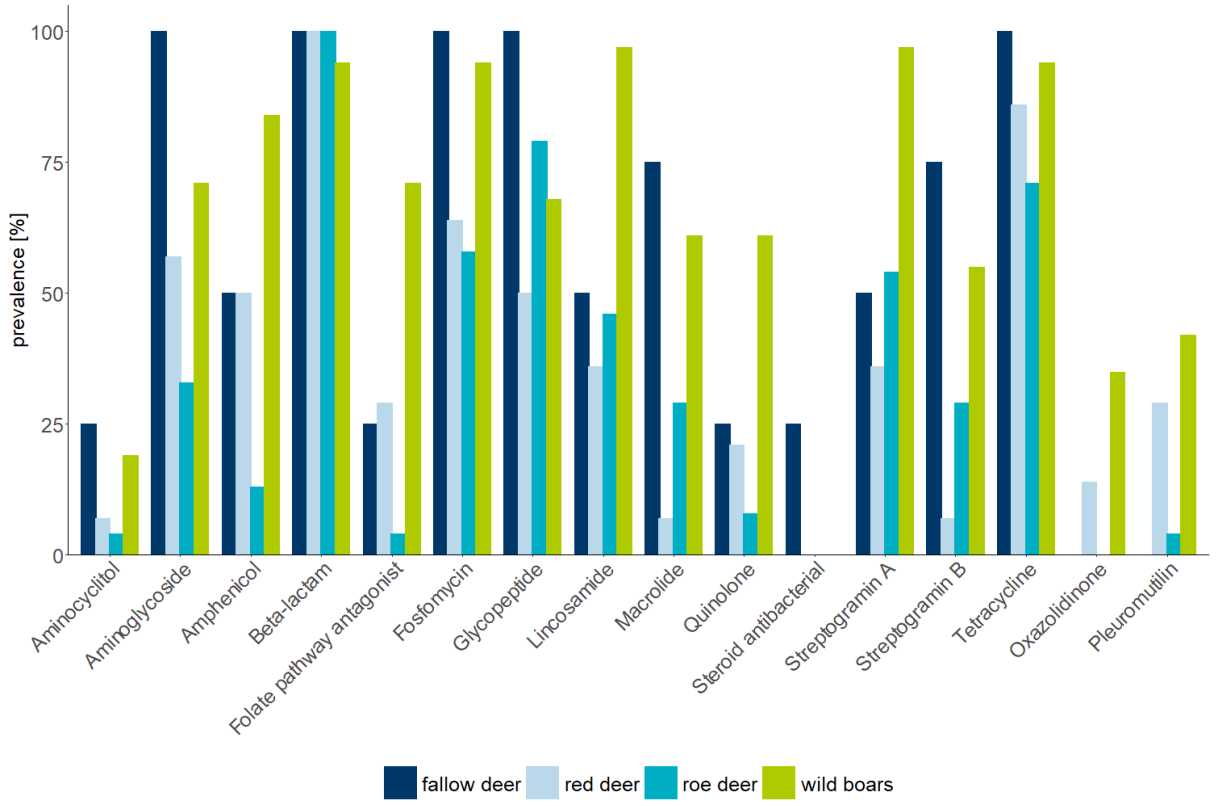
**Results:** In the hunting season 2024/2025, 73 pooled samples (wild boar n=31; roe deer n=24; red deer n=14; fallow deer n=4) derived from a total of 284 animals on 21 hunting days were investigated for the presence of AMR genes. In total, resistance genes against 16 antimicrobial classes were detected. Almost all samples (n=70) harbored resistance genes for beta-lactam antimicrobials, followed by resistance genes for tetracyclines and fosfomycin (Figure). Overall, wild boar harbored resistance genes for more classes of antimicrobials than the other species, with up to 15 classes of antimicrobials per sample. Further, more resistance genes per sample were detected in wild boar. Nevertheless, resistance genes were detected in all samples.

In 69 samples (95%) detected beta-lactam resistance genes were associated with cephalosporin resistance, with up to seven different extended-spectrum beta-lactamase (ESBL) genes detected in one sample from red deer and in one sample of wild boar. By excluding *cepA* (ESBL phenotype requires promoter alterations), 42 samples still harbored ESBL genes with $bla_{ACC}$ (n=19), $bla_{CMY}$ (n=18), $bla_{ACT}$ (n=12) and $bla_{DHA}$ (n=10) as the most abundant gene families. No CTX-M cephalosporinases were detected. Furthermore, five samples harbored carbapenemase encoding resistance genes belonging to the $bla_{OXA}$-,*cphA*- or *cfiA*-families.

**Conclusions:** Resistance genes were more abundant in samples from wild boar than from the other included species. This could be due to the fact that wild boar are omnivores, unlike the other wildlife species studied, or due to their proximity to human settlements and the possible transfer of resistance genes from humans and livestock into the environment. However, the most abundant ESBL family

detected in livestock and human was not detected, suggesting a limited anthropogenic impact on the observed patterns.

**Figure**




**OP-034**

# Assessment of Hygiene and Antimicrobial Resistance of *Enterobacteriaceae* in Crustacean Seafood, Mongers, and in-Contact Environment in Lagos State, Nigeria

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**Background and objectives:** Despite being an important animal protein source, crustacean seafood constitutes a significant source of foodborne infections. This study investigated crustacean seafood mongers' knowledge, attitudes, and practices on hygiene, food safety, and antimicrobial resistance (AMR) in Lagos, Nigeria. It also assessed the presence, and AMR profiles of *Enterobacteriaceae* in crustacean seafoods, on mongers" hands, and in the environment.

**Methods:** A cross-sectional survey was carried out using a semi-structured questionnaire to collect data on knowledge, attitudes and hygiene practices, as well as antibiotic usage, and knowledge of AMR from 121 crustacean seafood mongers. Biological samples including 121 crustacean seafood (comprising shrimps, prawns, crabs, and lobsters), 116 mongers" hand swabs, and 120 swabs of in-contact surfaces (bowls, trays, and tables) were obtained. Bacteria were isolated and identified by standard microbiological culture methods and biochemical characterization. Antimicrobial susceptibility testing was conducted by agar disk diffusion according to the recommendation of the Clinical and Laboratory Standards Institute. Isolates were also tested for phenotypic production of extended-

spectrum beta-lactamases (ESBL) using the cefotaxime/cefotaxime+clavulanic acid and ceftazidime/ceftazidime+clavulanic acid double disk combination test. Data were analyzed using descriptive statistics, correlation, and binary logistic regression.

**Results:** Many of the participants demonstrated poor knowledge (n=56, 46.7%) and negative/undesirable attitudes (n=43, 35.5%). Moreover, only 54 (44.6%) of the participants had good hygiene practices. Many mongers demonstrated good practices such as washing hands with soap after using the restroom (n=111, 91.7%). However, only 10 (8.3%) always washed their hands after handling money, and 14 (11.6%) after smoking, sneezing, or coughing. Also, only 35 (28.9%) always stopped handling seafood when sick or having diarrhoea. The factors significantly associated with respondents' hygiene practices were tribe (p = 0.021), location (p = 0.004), and form of seafood sold (p = 0.001). There was a significant negative correlation between participants" knowledge and practice (r = -0.185, p = 0. 001). The predictors of attitude and practice levels of the respondents were age (p = 0.039) and the form of seafood sold (p = <0.001) respectively. Furthermore, 19 (16.8%) of the respondents obtained their antibiotics by self-prescription, 23 (20.4%) from drug vendors (patent medicine stores) and four (3.5%) relied on advice from friends or family. In addition, 53 (46.9%) participants reported that they used antibiotics whenever they felt ill indicating a high prevalence of self-medication and inappropriate antibiotic use. Almost all participants (n=112, 92.6%) had no prior knowledge of AMR. The microbiological analyses revealed that 113 (97.4%) of the mongers" hand swabs, 116 (96.7%) of in-contact food surfaces" swabs, and 108 (89.3%) of crustacean seafood samples had bacterial contamination. *Escherichia coli* (n=53, 32.7%), *Enterobacter cloacae* (n=36, 22.2%), *Enterobacter agglomerans* (n=23, 14.2%), and *Citrobacter freundii* (n=23, 14.2%) were the most frequently isolated bacterial species. Overall, the isolates demonstrated resistance to ampicillin (n=123, 75.9%), cefoxitin (n=75, 46.3%), trimethoprim/sulfamethoxazole (n=48, 29.6%), and chloramphenicol (n=43, 26.5%). There were 75 antibiotic resistance phenotypes, 41 (55.5%) of which were multi-drug resistant (resistance to three or more classes of antimicrobial). Eleven (20.8%) out of the 53 *E. coli* isolates were phenotypically confirmed as ESBL-producers.

**Conclusions:** The findings emphasize the direct correlation between hygiene practices, the risk of dissemination of multidrug-resistant bacteria (including ESBL-producing *E. coli*) in the crustacean seafood mongering industry, and the significant knowledge gaps on AMR. It underscores the urgent need for improved hygiene practices, awareness of the risks of injudicious use of antibiotics, and better antimicrobial stewardship within the crustacean seafood supply chain.

**OP-035**

## Fate of Antibiotics in Cattle Manure and Their Impact on Nitrogen Cycling and Emissions

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**Background and objectives:** The widespread use of antibiotics in livestock production raises environmental and public health concerns, particularly through persistence of antibiotic residues in manure. These residues can alter microbial communities, select for antimicrobial resistance (AMR) and interfere with key biogeochemical processes such a nitrogen cycling. In manure, nitrogen (N) is a valuable nutrient for soil fertility but it"s production can lead to emissions of nitrous oxide ($N_2O$), a

potent greenhouse gas. This study investigated the fate of three commonly used antibiotics in cattle in Kenya—tylosin, enrofloxacin, and oxytetracycline—in manure, and their influence on microbial composition, and functional genes related to nitrogen transformation.

**Methods:** Fresh cattle manure (10 kg) was spiked with two concentrations (high and low) of each antibiotic, alongside an untreated control. The manure was incubated in open buckets for 110 days to simulate typical manure heaping conditions in Africa. $N_2O$ emissions were measured using a static chamber coupled with a laser spectrometer. Manure samples were collected at days 1, 21, 36, 54, 63, 89, and 110. Antibiotic residues were extracted using the QuEChERS method and quantified by LC-MS/MS. DNA was extracted using the Qiagen PowerSoil Kit and subjected to shotgun metagenomic sequencing on the NovaSeq X platform with a 150 bp read length.

**Results:** A progressive decline in antibiotic concentrations was observed over the 110- day incubation period, with tylosin, enrofloxacin, and oxytetracycline reduced by 99.0%, 83.4%, and 73.3%, respectively. Treatments with both high and low doses of tylosin, as well as the high-dose enrofloxacin, significantly increased $N_2O$ emissions (ranging from 2.10 ± 0.2% to 2.49 ± 0.4% of manure-N), exceeding the IPCC default emission factor of 1%. Given that nitrogen is essential for soil fertility and crop productivity, the elevated emission from manure is an environmental concern. Metagenomic analysis, currently underway, is expected to shed light on how antibiotic exposure may have disrupted microbial communities involved in nitrogen cycling—offering insights into the mechanisms behind altered emissions.

**Conclusions:** Manure storage alone is insufficient to fully eliminate antibiotic residues that contribute to global warming. These findings underscore the need for integrated AMR mitigation approaches that include improved manure management practice to minimize antibiotic contamination and reduce environmental impacts.

## *Session 5 - Mechanism and dissemination of antimicrobial resistance in animal and zoonotic pathogens*

### OP-036

## Genomic analysis of the *Staphylococcus pseudintermedius* mobilome associated with antimicrobial resistance

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**Background and objectives: Background and objectives:** The increasing antimicrobial resistance (AMR) in *S. pseudintermedius*, responsible for skin and soft-tissue infections (SSTIs) in companion animals, is a public health concern. The aim of this study was to analyze the carriage of mobile genetic elements (MGEs) and the associated AMR properties in *S. pseudintermedius*.

**Methods: Methods:** We studied 56 *S. pseudintermedius* representing predominant and emerging clonal lineages associated with SSTIs in dogs and cats collected in Lisbon (Portugal). These strains were subjected to plasmid DNA extraction and digestion with *Eco*RI and *Xba*I. Each unique restriction pattern was assigned to a plasmid profile. A subset of 15 strains was further selected for hybrid whole genome sequencing (WGS) (MinION and Illumina MiSeq).

**Results: Results:** Thirty-one of the 56 *S. pseudintermedius* strains carried one or more plasmid(s), mostly of small (≤ 5 kb) or medium (> 5 kb, ≤ 23 kb) size, corresponding to eight plasmid profiles. Only two of these plasmids carried AMR determinants; plasmid pSP-G3C4, isolated from ST71 strains, carried *tet*(K), while plasmid pSP5912, isolated from an ST2061 strain, harbored the *qacG* biocide resistance gene. Other AMR determinants were detected as part of MGEs integrated into the chromosomal DNA, namely Tn*552*, Tn*552*-like, Tn*553*, Tn*916*, Tn*5405*-like, Tn*5801*, Tn*5801*-like GI*6287* and pRE25-like element. In addition, a new chromosomal cassette, carrying *fusC*, was identified in a ST1183 strain. The 12 methicillin-resistant *S. pseudintermedius* studied carried SCC*mec* type III (n = 5), SCC*mec* type IVg (n = 3), SCC*mec*NA45 (n = 1), ΨSCC*mec*57395 (n = 1), the recently described SCC*mec*7017-61515 (n = 1), or SCC*mec* type V(T)SL/154 (n = 1). Ten strains carried intact prophages without AMR determinants. Intact restriction–modification systems were detected in 12/15 strains and CRISPR/Cas in 4/15 strains, three of which were methicillin-susceptible.

**Conclusions: Conclusions:** This study suggests that the AMR content in *S. pseudintermedius* is mainly associated with MGEs integrated into the chromosomal DNA rather than in plasmids. These results provide important insights that may lead to a better understanding of multidrug resistance in *S. pseudintermedius* towards improved SSTIs treatment in companion animals.

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**OP-037**

## Plasmids in *Staphylococcus aureus*: key vectors of antibiotic resistance genes dissemination

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**Background and objectives:** *Staphylococcus aureus* (SA) possesses a wide variety of antibiotic resistance genes (ARGs), and their dissemination is closely associated with the high proportion of mobile genetic elements (MGEs) present in SA genomes (between 15 and 20%). To date, few data are available on the diversity of ARG-carrying MGEs in SA, and their impact on the spread of antibiotic resistance is poorly documented.

**Methods:** An *in silico* analysis of 9054 human- and animal-associated SA genomes available in NCBI databases was carried out. An exhaustive mapping of associations between MGEs, ARGs and hosts was established using the ResFinder, RGI-CARD and MEFinder tools. In parallel, an *in vitro* analysis of 329 SA isolates collected from animals through the Resapath network between 2010 and 2021 and carrying ARGs was carried out. Plasmids were characterized by PFGE and the ARGs they carried determined by PCR. All identified plasmids were long-read sequenced (MinION) and a subset of 81 isolates were short-read sequenced (Illumina).

**Results:** *In silico* analyses of SA genomes identified 88 different ARGs and 305 different MGEs, including 167 plasmids (identified using the *rep* gene), 25 transposons and 14 integrative and

conjugative elements. Exploration of the MGE/ARG associations revealed that ARGs were predominantly present on plasmids (62%) and transposons (37%, with 2% associated with plasmids). Given their importance as ARG carriers, 211 plasmids collected from 329 SA isolates of animal origin in France over a ten-year period were characterized. The major families identified —*rep7a*, *rep20*, and *rep10*— were associated with specific resistance genes (*str*, *cat*, *blaZ*, *erm*(C)) and exhibited widespread horizontal transfer across different *SA* sequence types and animal hosts. Evolution of plasmids was observed, since the *rep7a*/str and *rep7a*/*cat* plasmids, circulating in horses, were progressively replaced by a *rep7a* plasmid carrying both *str* and *cat* genes. The study also highlighted the presence of mosaic (combining elements from different bacterial species/genera) and hybrid (displaying more than one *rep* gene) plasmids.

**Conclusions:** The diversity of MGEs and the predominance of ARG/plasmid associations confirmed that MGEs, and plasmids in particular, play a key role in the transmission of resistance in SA, whatever the host. It is therefore essential to characterize the transmission mechanisms of these elements in order to understand and better control the spread of antibiotic resistance in this major pathogen.

## OP-039

# Dissemination of Extended-Spectrum Beta-Lactamases producing *Escherichia coli* in poultry in Zimbabwe

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**Background and objectives:** Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* are resistant to the critically important third and fourth generation cephalosporin antibiotics and present a risk to animal and human health. In Zimbabwe there is an evidence gap concerning the prevalence and diversity of ESBL-producing *E. coli* in poultry.

**Methods:** In this study we screened for ESBL-*E. coli* at farms (n=50) and markets (n=10), according to methods published by the Fleming Fund. Isolates were examined by antimicrobial susceptibility testing and by whole genome sequencing using long-read and short-read methodologies. Geographic Information System mapping was used to visualise the distribution of the ESBL-producing clones.

**Results:** ESBL-*E. coli* were detected at every market and at 21 farms, giving a farm-level prevalence of 42%. A total of eight distinct *bla*CTX-M variants were identified and 69/70 (99%) isolates were multidrug resistant. Genomic analysis revealed evidence for clonal expansion of an ESBL-producing clone and horizontal gene transfer via plasmids being responsible for the dissemination of ESBL-*E. coli*. For example, ST1141 isolates were clonal, having a highly conserved core genome, and harboured *bla*CTX-M-15 and 11 additional antimicrobial resistance genes on a ~338kbp IncHI2 plasmid which was not present in other isolates. This clone was present at nine farms. In contrast, a conserved ~93kpb IncFII plasmid harbouring *bla*CTX-M-55 was present in isolates from three different Multilocus Sequence Types obtained from six farms.

**Conclusions:** This study addresses evidence gaps concerning the burden and distribution of ESBL-*E. coli* at poultry farms in Zimbabwe. The findings reveal clonal expansion and horizontal gene transfer as key drivers of ESBL dissemination, with evidence of a dominant ST1141 clone spreading across multiple farms. These results provide fresh insight into the understudied African poultry sector and

underscore the importance of adopting measures that can limit the development and dissemination of ESBL-producing *E. coli*.

**OP-040**

## Occurrence of multidrug-resistant *E. coli* and ESBL-producing *K. pneumoniae* in an organic broiler farm depends on chick origin

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**Background and objectives:** Recent studies suggest that hatcheries are a reservoir of antibiotic resistant strains and potential entry route into fattening flocks [1]. Multidrug-resistant (MDR) and ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* have been detected at different levels of the conventional broiler production pyramid [2,3]. The use of antibiotics on organic broiler farms is strictly limited, but both the prevalence and the source of resistant *Enterobacteriaceae* remain yet to be fully explored. This is of particular interest as the demand for sustainably and organically produced poultry meat is increasing.

**Methods:** Feacal samples were collected from four consecutive flocks at five time points over one year, covering the entire fattening period of the chickens starting from day-old chicks to slaughter age. Three *E. coli* and *K. pneumoniae* per sample were isolated on MacConkey agar alone and with 1mg/l cefotaxime for selective screening of ESBL-producing strains. All isolates were tested for antimicrobial susceptibility via broth microdilution. Whole Genome Sequencing was performed using both short-read (Illumina, NextSeq) and long-read sequencing (ONT, MinIon) for plasmid characterization and to reveal transmission events.

**Results:** Resistant *E. coli* and ESBL-producing *K. pneumoniae* were frequently isolated from the first three flocks (66.7%, resp. 78.6%) already from samples of day-old chicks and throughout the fattening period. All ESBL-producing *K. pneumoniae* belonged to a clonal ST307 strain with a conjugative IncFII/IncFIB plasmid harboring *bla*CTX-M-15, *bla*TEM-1, *aph(6)-Id*, *aph(3'')-Ib*, *sul2*, *dfrA14* and *aac(3)-IIa* resistance genes. Compared to *K. pneumoniae*, the resistant *E. coli* population of flocks 1-3 showed an overall high genetic diversity with 15 different MLST. We also found a distinct cluster of clonal MDR ST162 (15.6%, 7/45) re-appearing in flocks 1-3 harboring *aadA1*, *aadA2*, *aac(3)-IVa, aph(4)-Ia*, *bla*TEM-1B, *cmlA1*, *sul3* and *tet(A)* resistance genes on a conjugative IncFII/IncFIB plasmid. Chicks for flock 4 were purchased from a different organic hatchery than flocks 1-3. From this flock, only few resistances and no ESBL-producers were found. The majority of *E. coli* and *K. pneumoniae* isolates were fully susceptible (66.7% resp. 100%). Therefore, an organic certified hatchery supplying chicks for flocks 1-3 was likely to be the source of the MDR strains.

**Conclusions:** Our findings highlight the importance of chick origin for introducing antimicrobial resistant ESKAPE(E) pathogens into organic broiler farms. Future studies targeting organic parent flocks and hatcheries are needed to evaluate the impact of possible transmission events and to develop targeted interventions if needed.

**OP-041**

# Plasmid-chromosome interactions regulate the conversion of the tigecycline resistance phenotype in *Klebsiella pneumoniae*

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**Background and objectives:** *Klebsiella pneumoniae* is an increasingly important bacterial pathogen that can cause severe organ infections and pose a significant threat to human life. In recent years, carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-HvKP) isolates have emerged, which are capable of causing severe infections [1]. Tigecycline has been considered the last resort of defense for treating CR-HvKP. However, the emergence of tigecycline resistance poses a significant risk of treatment failure [2].In this study, we identified that an IS*26*-mediated insertional mutation in *ramR* due to the plasmid-chromosome interaction which contributes to tigecycline resistance by regulating the overexpression of the AcrAB-TolC efflux pump in *K. pneumoniae*.

**Methods:** A comprehensive analysis of 1,439 whole-genome-sequenced *K. pneumoniae* strains was performed to identify single nucleotide polymorphism (SNP) variants in the *ramR* gene. The effect of tigecycline exposure on *ramR* expression, impact of *ramR* on AcrAB-TolC efflux pump expression, and the effect of overexpression of the AcrAB-TolC efflux pump on tigecycline resistance were evaluated to clarify the mechanism of plasmid-chromosome interaction in regulating the conversion of the tigecycline resistance phenotype through bioinformatics, CRISPR knockout, and RT-qPCR. The minimal inhibitory concentrations (MICs) were determined by broth dilution methods.

**Results:** Of 1,439 *K. pneumoniae* isolates, 76.7% harbored a SNP at the *ramR* stop codon (*194K), with 18.1% a W185* mutation, and 2.3% an A19V mutation. Introduction of plasmids carrying mutant *ramR* variants into wild-type strains significantly increased the tigecycline MICs. During tigecycline exposure, the two plasmids in *K. pneumoniae* YZ6 underwent homologous recombination, resulting in the formation of a fused 230 kb segment, which subsequently inserted into the chromosomal *ramR* gene via IS*26*, then led to a significant increase in tigecycline resistance.

**Conclusions:** Mutations in *ramR* which enhance tigecycline resistance by inactivating the negative regulatory function of the AcrAB-TolC efflux pump are highly prevalent in clinical *K. pneumoniae* strains. Furthermore, the discovery of plasmid fusion and IS*26*-mediate plasmid-chromosome integration events reveals a novel pathway for the dynamic evolution of bacterial resistance through mobile genetic elements.

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**OP-042**

# The interplay between heteroresistance and resistance: gene duplication-amplification delays stable population-wide resistance through clonal interference

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**Background and objectives:** Heteroresistance (HR) describes a phenomenon of transient resistant subpopulations within a susceptible population that grow in the presence of inhibitory antibiotic concentrations. The detection of HR in standard diagnostics is insufficient, which leads to clinical complications. Moreover, HR can pave the way to stable population-wide resistance. We detected HR to ceftazidime (CAZ) in a clinical *Enterobacter cloacae* complex (ECC) strain (IMT49658).

**Methods:** We performed extensive phenotypic (population analysis profiles, stability analysis of resistance, ScanLag) and molecular microbiological techniques (qPCR, whole genome sequencing, raw read analysis) to demonstrate the mechanism of HR and its impact on the phenotype of resistant subpopulations. We investigated the transition from HR to stable resistance in two evolution experiments with a) long-term CAZ pressure for 21 days and b) overnight exposure to high CAZ concentrations, accompanied by profound fitness analysis (competition assays) and deterministic population modelling.

**Results:** WGS and qPCR detected a plasmid borne gene duplication-amplification (GDA) with an ampC β-lactamase *bla*DHA-1 of high copy number in resistant subpopulations. These decreased when grown without antibiotic, leading to susceptibility. In ScanLag, growth of resistant subpopulations showed heterogeneous lag times in direct correlation with their GDA copies. GDAs varied between colonies, but also within single cells of a colony (raw read analysis). Evolution experiments in long-term or high antibiotic pressure led to the transition from HR to stable resistance *via* mutation based ampC-derepression. Deterministic modelling of population dynamics showed that the emergence of mutants in GDA-containing resistant subpopulations depends on fitness costs and frequency of both GDAs and mutants. GDAs rescued small populations under high antibiotic pressure but also delayed newly emerging mutations.

**Conclusions:** We broadened the perspective of GDA-driven HR as a pre-step of stable population-wide resistance. Depending on population size, fitness of concurrent resistance mechanisms and forms of antibiotic pressure, GDAs can rescue bacterial populations or delay stable, non-costly resistance. These observations are of great value for understanding the transition from HR to resistance in an ongoing antibiotic crisis.

**OP-043**

## Genomic characterization of 2 clonal lineages of *E. coli* (ExPEC) multi drug resistant responsible for septicemia in Quebec neonatal calves

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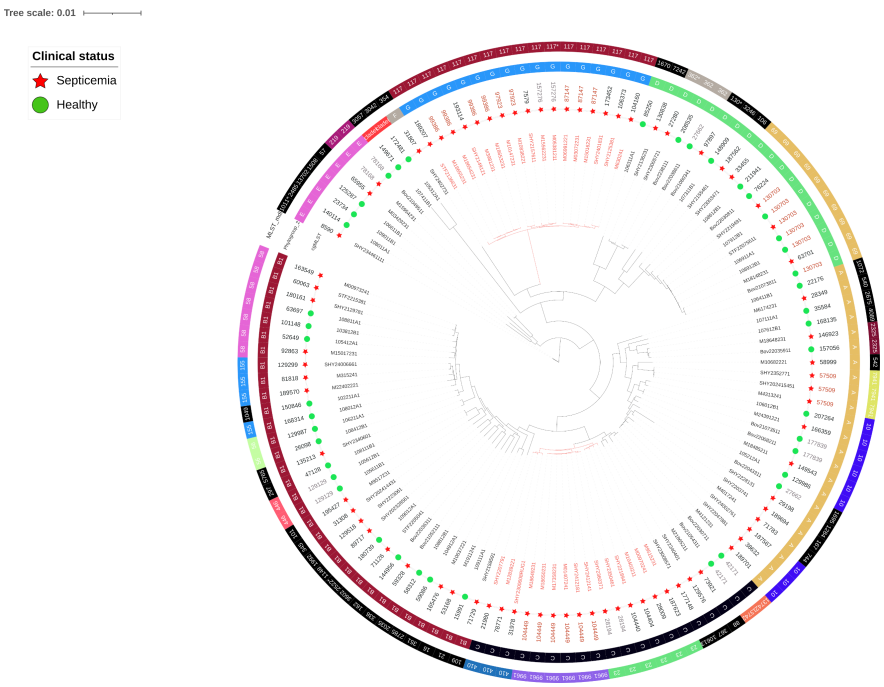
**Background and objectives:** Over the past 5 years, we have observed an increase in mortality among neonatal calves due to *E. coli* infections, and this phenomenon does not seem to be restricted to Quebec [1]. However, these strains lack the virulence factors routinely identified which complicates their diagnosis. In addition, over 90% of these strains are multi-drug resistant (MDR). Their resistance to antibiotics critical to human medicine (3rd generation cephalosporins (C3G)) is also increasing.

**Methods:** A total of 120 *E. coli* isolates have been sequenced. Of these, 42 isolates are considered commensal and were isolated from manure of healthy calves under 2 months of age, while 78 of these isolates originate from necropsies of calves presenting clinical signs of septicemia and were isolated at the CDEVQ Bacteriology Laboratory. Complete genome sequencing was performed. The Center for Genomic Epidemiology (CGE) platform was used for *in silico* analysis (FimTyper, PlasmidFinder, MLST, ResFinder, virulenceFinder and SeroTypeFinder).

**Results:** We have identified 2 clonal lineages responsible for 50% of cases. Isolates from the first clonal lineage belong to phylogroup G, ST117 and various O:H4 serotypes. Isolates from the second clonal lineage belong to phylogroup C, ST410/ST23/ST9961 and various O:H9 serotypes. The phylogenetic characteristics of the other half of the pathogenic isolates are diverse. Some[SB1] of the isolates associated with sepsis possess avian pathogenic *E. coli* virulence genes (APEC-like), which are most often disseminated by an IncFIA plasmid, or genes identified as encoding components of the CS31A fimbriae (*faeC, faeD, faeE, faeF, faeI*), which are probably disseminated by another plasmid, IncFIB. These plasmids are present in some sepsis-associated isolates but have not yet been fully characterized. **Two plasmids, IncHI2 and IncI, known to carry multiple resistance genes, are also present in some**[SB2] **of the isolates associated with sepsis**. We had previously found the same IncHI2 plasmid in commensal isolates carrying *qnrS1* and *blaCTX-M-55*, conferring resistance to fluoroquinolones and (C3G) respectively.

**Conclusions:** The 2 clonal lineages responsible for half the cases are potential candidates for a vaccine. Our results illustrate the complex relationship between pathogenic and commensal isolates and underline the importance of monitoring healthy microbiota to explain antimicrobial resistance in clinical cases.

**Figure**



**OP-044**

# Detection of antibiotic resistance genes in food from aquaculture

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**Background and objectives:** The worldwide demand for fish is rising and cannot be met by fish form wild catches in a sustainable way. Aquaculture makes it possible to produce large amounts of fish in small areas. Open aquacultures are known for years and antimicrobials are used to treat and prevent infections usually in large scale. Closed aquacultures like recirculating aquaculture systems (RAS) and aquaponics recently gained popularity and in contrast to open aquacultures it is not feasible to use antibiotics. Little is known about the resistome (all resistance genes within a sample) in RAS/aquaponic settings. Analysing the resistome in food samples can be done by shot gun metagenomic sequencing. Targeted enrichment as a quasi-metagenomic approach is a cost and time effective option for the detection of resistance genes in food samples (Beaudry et al., 2021).

**Methods:** In our study at total of 84 samples were analysed with 27 samples coming from RAS/aquaponics and 57 samples from open aquaculture originating from different countries in Europe. Different species of fish and shrimps were purchased from supermarkets, online shops or directly from the manufacturer. Upon arrival, the fish was mixed with buffered peptone water followed by incubation at 37°C for 18-24 hours. The DNA was extracted from 1 ml of the overnight enrichment culture using ZymoBIOMICS DNA miniprep. The library preparation for sequencing was performed using Illumina DNA Prep, (M) Tagmentation (96Samples) and Primer IDT® for Illumina Nextera DNA Unique Dual Indexes. Resistance genes were enriched using a bait-based hybridization capture enrichment kit (AMR-Cap-Antimicrobial Resistance Gene enrichment panel, myBait, Daicel arbor biosciences, which was followed by NextSeq (illumina) sequencing. Trimmed raw reads were analysed for resistance genes using ResFinder 4.0 (identity and coverage of 90 %).

**Results:** The results showed a total number of 163 resistances genes for 17 different antibiotic classes, yet no sample was positive for all 17 antibiotic classes. In four samples resistance genes against 14 different antibiotic classes were found. All analysed samples (n=84) were positive for at least one gene for the resistance against beta-lactam antibiotics, followed by tetracycline (n=80), amphenicol (n=75), and quinolone (n=72), which are also the antibiotics mostly used in conventional aquaculture.

Resistances against beta-lactam antibiotics, especially resistances against carbapenem are an increasing thread to human health as carbapenem antibiotics are used as last resort antibiotics. In 25 (92.6%) samples from RAS/aquaponics and in 54 (94.7%) samples open aquaculture, genes responsible for the resistance against carbapenem antibiotics were found. The genes *bla*OXA(n=18 and n=38), *cphA* (n=19 and n=54), and *cphA1* (n=17 and n=53) were detected in both RAS/aquaponics and open aquaculture, respectively. In RAS/aquaponics *bla*B, *bla*EBR, *bla*GOB, *bla*IND, *bla*MUS and in open aquaculture *bla*VCC and *bla*VIM were detected, respectively. It is to note that the genes *bla*EBR, *bla*GOB, *bla*IND, *bla*MUS were detected in the same sample from RAS.

**Conclusions:** Even though, in closed aquacultures no antibiotics are used, a wide variety of antibiotic resistance genes can be found in samples from fish from closed aquaculture settings. This include antibiotics that are used as last resort antibiotics. The entry route and transferability of resistance genes between different bacterial species in closed aquaculture settings is yet to be determined and needs further investigation.

Beaudry M. S., et al., 2021,"Escaping the fate of Sisyphus: assessing resistome hybridization baits for antimicrobial resistance gene capture," Environ Microbiol, **23**(12), pp. 7523–7537. doi:10.1111/1462-2920.15767.

**OP-045**

# Impact of an oxytetracycline administration *per os* on the intestinal microbiota and resistome in pigs

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**Background and objectives:** In veterinary medicine, collective antibiotic treatments are often administrated orally. However, as the drug passes through the digestive tract, it affects pathogenic bacteria and the commensal microbiota, potentially promoting antibiotic resistance. After oral administration, a fraction of the antibiotic binds to the digestive matrix, while the free fraction exerts selective pressure, favoring resistance. This study aims to characterize the impact of oxytetracycline (OTC) on the microbiota and resistome across different digestive segments in pigs. Additionally, it seeks to predict the free fraction of OTC in the intestine following oral administration.

**Methods:** Eight piglets received a single OTC dose (20 mg/kg bw) *per os*. After euthanasia, contents from the jejunum, cecum and rectum were collected at six hours ($n$ = 4) and 24 hours ($n$ = 4) post-treatment. A control group of four untreated animals was included. Tetracycline resistance genes were quantified using qPCR, while microbial diversity was assessed by sequencing the V3-V4 region of the 16S rRNA gene. Finally, OTC concentrations in different digestive segments were determined by UPLC-UV. As a fraction of OTC binds to the intestinal matrix, reducing the active fraction, its antimicrobial activity was assessed against an *Escherichia coli* strain in sterilized intestinal contents and compared to its activity in Mueller-Hinton broth.

**Results:** The study found no significant differences in the quantity of OTC-resistant genes in the microbiota at six or 24 hours post-treatment, nor in microbial diversity or absolute abundance, compared to the control group. This stability may be attributed to the low exposure time. Furthermore, OTC was detected in the jejunum and cecum six hours after administration, and only in the cecum (at lower concentrations) and rectum after 24 hours. Moreover, time-kill curves revealed a progressive reduction in OTC activity along the digestive tract, with even greater decreases when compared to the standard culture broth.

**Conclusions:** This study provides new insights into the impact of OTC on the intestinal microbiota and resistome in pigs. Despite no significant short-term changes in microbial diversity or the abundance of OTC-resistant genes, time-kill curves demonstrated a progressive reduction in OTC activity along the digestive tract, highlighting the importance of considering both total and active antibiotic concentrations when evaluating antimicrobial pressure on gut bacteria. Using this pharmacokinetic-pharmacodynamic approach, we propose a preclinical pig model to assess intestinal microbiota exposure to OTC while accounting for intestinal contents. In perspective, we seek for improving this model and integrate the digestive microbiota.

## *Session 6 - Novel approaches to combat antimicrobial resistance*

**OP-046**

# New candidate drugs for repurposing as efflux inhibitors and antibiofilm agents against antimicrobial-resistant *Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis*

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**Background and objectives:** *Staphylococcus aureus* (SA), *Staphylococcus epidermidis* (SE), and *Enterococcus faecalis* (EF) are relevant pathogens, frequently associated with chronic infections resilient to chemotherapy. This resilience may result, partially, from efflux-mediated resistance and biofilm formation. The lack of options to treat such infections emphasizes the need to find new alternative therapeutics. In a previous *in silico* drug repurposing study, we identified over 200 drugs potentially targeting staphylococcal transporters, including efflux pumps, and proteins involved in biofilm formation. In this study, we aim to assess the potential for repurposing as efflux inhibitors and/or antibiofilm agents of a subset of 57 candidate drugs of different chemical classes.

**Methods:** Efflux and biofilm inhibitory activities were tested in isogenic and reference SA or SE strains, differing in the expression of *norA* gene (encoding NorA, a main efflux pump), and in clinical strains with relevant traits (clonality, resistance, biofilm production). The efflux inhibitory (EI) activity was assessed by the drugs" ability to modulate MICs of effluxable antimicrobials and fluorometry. Drugs showing the most significant effect [modulation factor (MF) score ≥ 4 and/or relative final fluorescence value (RFF) ≥ 1], were further studied by checkerboard assays to determine the lowest effective concentrations. The drug"s ability to inhibit biofilm formation or eradicate mature biofilms was tested by the crystal violet adhesion method and minimum biofilm eradication concentrations (MBEC) assays, respectively. Drugs with efflux inhibitory activity were further tested against EF reference strains. The *in vivo* efficacy of the drugs is under assessment, individually or in combination with ciprofloxacin, in a *Galleria mellonella* infection model.

**Results:** Of the 57 drugs tested, only two presented mild antimicrobial activity (MICs 8 – 32 mg/L). Twenty-five drugs showed potential as efflux inhibitors (significant MIC decrease of NorA substrates and RFF ≥ 1 in *norA*-overexpressing strains). Seven drugs presented dual EI and antibiofilm activity in both staphylococcal species, at concentrations of 16 – 1024 mg/L. Two drugs showed dual activity only in *S. aureus* and other two in *S. epidermidis* only. One of the most promising drugs was able to eradicate *S. aureus* mature biofilms. From the 25 potential efflux inhibitors identified above eight showed efflux inhibitory activity also in EF (MF ≥ 4 and/or RFF ≥ 1), five of which with dual EI and antibiofilm activities. Ongoing infection assays indicate that top candidate drugs have a mild effect at high doses to treat infections caused by these bacteria in *G. mellonella*.

**Conclusions:** This study highlights the potential of clinically approved drugs for repurposing as efflux and biofilm inhibitors to treat infections caused by antimicrobial-resistant Gram-positive pathogens.

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**OP-047**

# Colanic Acid-Mediated Phage Resistance Enhances Virulence in High-Risk *Escherichia coli* ST410

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**Background and objectives:** *Escherichia coli* ST410, a multidrug-resistant high-risk clones, demonstrates pan-ecological colonization competence across human, veterinary (companion animals/livestock), wildlife, and environmental reservoirs[1]. Its concurrent evolution of hypervirulence and antimicrobial resistance profiles presents critical challenges for clinical management, with phage therapy emerging as a prominent candidate[2]. This study focuses on isolating phages targeting *E. coli* ST410, elucidating the mechanisms of phage resistance, and exploring phage therapy strategies.

**Methods:** The carbapenem-resistant *E. coli* ST410 JXZ9A32M was used to isolate phage. Whole-genome sequencing was followed to analysis the mutations of phage-resistant bacteria occurred via phage treatments in *vivo* and in *vitro*. Genetic complementation was used to verify the relationship between the mutation sites and phage resistance (high colanic acid production). Virulence of resistant strains was assessed through macrophage phagocytosis resistance assays and murine infection models.

**Results:** A sewage-derived phage P32M-3-Y demonstrating lytic activity against ST410 B5/H24RxC and B3/H24Rx strains carrying Onovel1 was isolated. Resistant mutants from high-MOI cultures and murine models harbored O-antigen biosynthesis gene mutations, reducing phage adsorption. A hypermucoid mutant (32M3BB) exhibited a Y97N substitution in *yrfF* which is RcsCDB phosphorelay repressor. Complementation of this mutant with the wild-tye *yrfF* could restore its morphology and phage sensitivity. Transcriptomics also revealed that this mutation led to upregulation of *rcsA* and colanic acid synthesis genes. Notably, this mutant displayed enhanced virulence, achieving 70% murine mortality within 24h, coupled with 20-fold reduced macrophage phagocytosis. This finding suggests that phage resistance mutations in ST410 can, to some extent, promote virulence evolution. To address rapid phage resistance development and potential virulence enhancement in resistant strains, we conducted a second round of phage screening for resistant strains and designed a cocktail of four phages whose therapeutic activity was confirmed.

**Conclusions:** This study establishes a phage-based therapeutic alternative for carbapenem-resistant high-risk *E. coli* ST410, while providing the first mechanistic evidence that phage resistance evolution drives hypervirulence through colanic acid-mediated immune evasion.

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**OP-048**

# Anti-bacterial Monoclonal Antibodies for Precision Diagnostics and Targeted Therapy

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**Background and objectives:** The rise of multidrug-resistant (MDR) bacterial pathogens presents a significant global health threat, undermining decades of medical progress. This "antibiotic resistance crisis" has been driven by the widespread—and often inappropriate—use of broad-spectrum antibiotics, compounded by a decline in the discovery of new antimicrobial agents. As resistance outpaces drug development, innovative alternatives to traditional antibiotics are urgently required. Monoclonal antibodies (mAbs) offer a promising solution due to their high specificity for bacterial virulence factors, enabling targeted therapeutic interventions and reducing reliance on broad-spectrum agents. Our objective is to discover, develop, and optimize anti-bacterial mAbs that can serve a dual role: as therapeutic agents to inhibit key virulence mechanisms, and as precision diagnostic tools within point-of-care (POC) platforms for rapid and accurate pathogen detection and antibiotic resistance profiling.

**Methods:** We employ phage and yeast surface display libraries of single-chain variable fragments (scFvs) to screen for mAbs targeting antibiotic-resistant bacterial pathogens. Selected scFv candidates are cloned and expressed as recombinant mAbs. These antibodies are then either incorporated into electrochemical biosensors for diagnostic applications or developed as therapeutic leads.

**Results:** We have identified a mAb that targets the Type III Secretion System (T3SS) of enteropathogenic *Escherichia coli* (EPEC), a key virulence mechanism. This antibody exhibits high affinity and specificity. It was successfully integrated into an electrochemical sensor, demonstrating sensitive and accurate detection capabilities [1]. The biosensor was further optimized into a dual-channel format, enabling simultaneous detection of pathogenic bacteria and their antibiotic resistance profiles [2]. Moreover, the mAb showed promising therapeutic potential in neutralizing T3SS activity, suggesting utility in treating bacterial infections.

**Conclusions:** Our findings demonstrate the potential of anti-bacterial mAbs as both precision diagnostics and targeted therapeutics. Integration into POC platforms supports rapid, pathogen-specific diagnostics, while therapeutic application offers a novel approach to infection control. Collectively, these strategies could significantly reduce dependence on traditional antibiotics and play a critical role in addressing the global challenge of antimicrobial resistance.

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**Figure**

**OP-049**

# Metabolites produced by gut bacteria under anoxic conditions drive the suppression of *Acinetobacter baumannii*

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**Background and objectives:** *Acinetobacter baumannii* is a multidrug-resistant pathogen that poses a significant threat in healthcare settings [1] due to its ability to colonize the gastrointestinal (GI) tract and form reservoirs [2], thus increasing the risk for systemic and polymicrobial infections [3]. This

study investigates how *Klebsiella oxytoca*, a gut commensal known for its protective effects against pathogens, interacts with carbapenem-resistant *A. baumannii* (CRAB) under varying environmental conditions.

**Methods:** Coculture experiments were conducted to assess the interaction between *K. oxytoca* and CRAB under various environmental conditions, including oxygen levels, carbon source availability, and environmental pH. The experiments also involved screening for enteropathogens and intestinal commensal strains, while excluding cell-cell contact dependency and bacteriocins. Additionally, cross-feeding experiments provided insights into broader metabolic network possibilities.

**Results:** We found that specific carbohydrates confer a competitive advantage to *K. oxytoca*, enabling it to suppress *A. baumannii* through secreted metabolites and environmental modulation. This suppression occurs through multifactorial mechanisms independent of *Klebsiella* toxins and is supported by other members of the gut microbiome. Additionally, *A. baumannii* displayed survival and limited growth under anoxic and hypoxic conditions, demonstrating its adaptability within intestinal niches.

**Conclusions:** These findings indicate that targeted carbohydrate supplementation and innovative probiotic strategies may enhance the metabolic versatility of *K. oxytoca*, fostering an inhospitable environment for *A. baumannii*.

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# Poster Presentation

## *t01 – Monitoring and molecular epidemiology of antimicrobial resistance*

### P-004

## Bacterial pathogens from small animal blood cultures and their antimicrobial resistances (2014-2022)

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**Background and objectives:** Sepsis is a prevalent and life-threatening condition [1]. Antimicrobial therapy is the cornerstone for the treatment of bacterial sepsis [2]. Blood cultures (BCs) are the gold standard for the diagnosis of bacterial sepsis and are integral for the selection of an appropriate antimicrobial therapy [3]. Hence, bacterial isolates from positive BCs obtained from cats and dogs and their antimicrobial resistance profiles were investigated in order to gain an overview of the isolates and resistance patterns present in our clinic.

**Methods:** In this single-center study, microbiological BC results from cats and dogs presented in a veterinary teaching hospital (2014-2022) were analyzed retrospectively with Microsoft Excel® and Graphpad Prism®.

**Results:** Clinically relevant growth was isolated in 102/750 BCs (13.6%) from dogs and in 10/150 BCs (6.6%) from cats. The contamination rates were 1.9% and 4,0%, respectively. In dogs, the most common isolates from monobacterial BCs were Enterobacterales (n=22), coagulase-positive *Staphylococcus* spp. (n=21), beta-hemolytic *Streptococcus* spp. (n=17), and obligate anaerobic species (n=16). There was polymicrobial growth in eight BCs. In cats, the bacteria isolated were Enterobacterales (n=5), *Pasteurella* spp. (n=3), *Enterococcus* sp. (n=1), and in one polymicrobial BC, *Enterococcus faecium* and *Enterobacter cloacae*. In 30 of the 102 BCs of dogs, multidrug-resistant (MDR) pathogens were isolated. Of the Enterobacterales isolates, 60% were MDR. Three of the *Staphylococcus pseudintermedius* isolates were methicillin-resistant. In cats, five (*Escherichia coli* [n=2], *Enterobacter cloacae* [n=2], *Enterococcus faecium* [n=1]) MDR pathogens were isolated.

**Conclusions:** The reported antimicrobial resistances prove the importance of microbiological diagnostics of BCs in patients with bacterial sepsis to guide antimicrobial therapy and their further resistance monitoring.

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## P-007

# Genetic analysis of cefotaxime-, fluoroquinolone- and colistin-resistant *E. coli* from veal calves up to eight months of age

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**Background and objectives:** *Escherichia coli* (*E. coli*) is used as an indicator bacterium for surveillance to monitor the colonization of livestock animals with antibiotic resistant bacteria. In Germany, half of the tested commensal *E. coli* isolates derived from the cecum of veal calves are resistant to at least one antimicrobial class [1]. However, the currently applied monitoring program

leaves aside the variety of different production systems for veal calves and is based on unselective laboratory methods. We investigated fecal samples of veal calves on resistances to highest priority critically important antimicrobials using selective laboratory methods and addressing different production systems (closed farming systems of dairy farms (A), farms puchasing calves <10 weeks of age (B) or >10 weeks of age (C), suckler cow herds (D) and traders (E)).

**Methods:** Two pooled fecal samples of veal calves, representing the oldest and youngest group on each farm, were collected from 81 farms (D and E one sample each) and screened for phenotypic resistant *E. coli* on MacConkey agar supplemented with cefotaxime (CTX), enrofloxacin (ENR) or colistin (COL). Up to three distinct colony morphologies per sample and supplemented antibiotic were genotyped using pcr and Sanger sequencing. Analyses included mobile ESBL-/ pAmpC- genes (*bla*CTX, *bla*SHV, *bla*TEM, *bla*CMY), mobile colistin resistances (*mcr-1* to *-5*), chromosomal mutations in *gyrA* and *parC* and mobile fluoroquinolone resistances (*qnr*, *qepA*, o*qxA/B*, *aac(6")-Ib-cr*).

**Results:** High prevalence of CTX and ENR resistant *E. coli* (>70%) were detected in A, B and C farms and traders (E). Suckler cow herds (D) showed low prevalence to all tested antibiotics (<25%). The CTX and ENR prevalence of younger calves was higher compared to older calves in A, B and C farms. When both samples from a farm were positive for CTX- or ENR-resistant *E. coli*, the genetic diversity between the samples was highest in B farms and lowest in A farms. Prevalence of COL resistant *E. coli* was lower (< 20%) with detection of *mcr-1*. Most frequently detected Beta-Lactamases were CTX-M-1, -15 and -14. Most frequent mutations in ENR resistant *E. coli* were S83L, D87N/Y/H and S80I and detection of mobile resistance genes *qnrS* and *aac(6")-Ib-cr*.

**Conclusions:** Veal calves of different production systems in Germany showed high prevalence to highest priority critically important antimicrobials. Only suckler cow herds showed lower prevalence in our study. Differences in the prevalence and genetic diversity of the production systems were found, especially in young calves, supporting further differentiation of species in surveillance programs based on production system to improve antibiotic resistance monitoring.

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## P-008

# Prevalence and Genetic Correlation of Linezolid-Resistant Enterococci on Swine Farms in South Korea

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**Background and objectives:** Linezolid (oxazolidinone) is used as the last-resort treatment for vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus* in clinical settings. Despite not being used in livestock, concerns have arisen due to the spread of transferable resistance genes in animals. This study aimed to evaluate linezolid resistance in enterococci isolated from swine farms in South Korea comparing these strains with human isolates.

**Methods:** Fecal samples were collected from pigs (n=600), farm environments (n=300), and workers (n=45) across 15 pig farms in South Korea. The strains isolated on Enterococcosel agar were analyzed for antibiotic susceptibility, oxazolidinone/phenicol resistance genes, and genotyping. Whole-genome sequencing was performed through hybrid assembly of long- and short-read sequences generated by next-generation sequencing platforms.

**Results:** A total of 1,085 *Enterococcus faecalis* strains (766 from pigs, 268 from the farming environment, and 51 from farm workers) and 313 *E. faecium* strains (190 from pigs, 77 from the farming environment, and 46 from farm workers) were collected. The linezolid resistance phenotype was observed in 169 *E. faecalis* strains (15.6%) and 2 *E. faecium* strains (0.6%). No mutations

associated with linezolid resistance were found in the 23S rRNA gene sequences of all linezolid-resistant enterococci (LRE). However, linezolid resistance genes were detected in a total of 176 *E. faecalis* strains (including 7 non-resistant strains). Of these, 163 strains harbored only *optrA* gene. Eight strains were positive for *optrA*/*cfrD*, and three strains contained *optrA*/*poxtA*/*cfrD*. Additionally, three strains possessed *poxtA*/*cfrD*, and one strain had only *cfrD*. The two linezolid-resistant *E. faecium* strains harbored *poxtA*. Linezolid-resistant *E. faecalis* (LREfs) were isolated with higher prevalence in suckling pigs (n=29) and weaned pigs (n=41) than in growing pigs (n=22) and finishing pigs (n=18) during the rearing stages. Among all LREfs, the most dominant sequence type (ST) was ST476 (30.2%, 51/169), and these strains mostly harbored *optrA* as well as the phenicol resistance gene *fexA*. High correlations were observed among *E. faecalis* ST620 strains isolated from workers, pigs, and the environment of the same or different farms. These clones harbored plasmids (approximately 92 kb) that included multidrug resistance (MDR) genes.

**Conclusions:** LRE in swine farms was detected more frequently in young pigs and their habitats with a higher proportion among the collected specimens. And *E. faecalis* ST620 clones found in various farming ecosystems contained plasmids with MDR.

## P-009

# Exploring Antimicrobial Resistance in Environmental Bacteria Collected by Honey Bees Through a One-Health Approach

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**Background and objectives:** Antimicrobial resistance (AMR) represents a significant global health threat and understanding the factors driving the spread of resistance among environmental microorganisms within a One-Health framework is crucial. Honey bees (*Apis mellifera* L.) forage over large areas, with their hairy bodies trapping environmental particles, including bacteria and contaminants. We hypothesized that these foragers may act as trapping vectors of AMR, behaving as bioindicators of the surrounding environmental resistome diversity. This study aimed to characterize and assess the presence of AMRs in the environmental bacteria strains collected by honey bees from different habitats in South Tyrol (Italy).

**Methods:** Sampling was conducted across different seasons (spring, summer, autumn) and habitats (urban, agricultural, natural, and livestock-dominated areas). Microorganisms isolated from honey bees" gut and surface were identified using MALDI-TOF mass spectrometry. The presence of AMRs was assessed phenotypically by MIC tests and genotypically by multiplex PCR, for each isolate.

**Results:** A total of 261 isolates were obtained, revealing a noteworthy microbial diversity among the samples. Of the 99 identified species, 53% were isolated only once. Among these species were various plant and animal pathogens, including *Pantoea agglomerans*, *Bacillus cereus*, and *Pseudomonas aeruginosa*. *Pantoea agglomerans* was isolated 27 times, accounting for approximately 10% of all isolates, with a widespread distribution across different seasons and sampling sites. *Bacillus cereus* was identified in 20 isolates, representing 7% of the total. Most isolates were resistant to at least one antimicrobial class, and many were multidrug-resistant, including resistance to colistin and the presence of *tetM* and *tetC* genes.

**Conclusions:** This study highlights how geographic and seasonal factors may influence the variety of environmental strains collected by honey bees and the prevalence of antimicrobial resistances. These findings confirm the potential of honey bees as bioindicators of environmental AMR. In future studies, we will focus on the understanding of the mechanisms of antibiotic resistance gene transmission within and between ecosystems to better assess the ecological impact of pollinator-mediated resistance spread.

**P-010**

# A Two-Hour Microfluidics-Based Detection Workflow for Concurrent Detection and Identification of Common Upper Respiratory Pathogens

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**Background and objectives:** Timely detection and accurate identification of infectious pathogens are key in developing tools used by public health programs to conduct outbreak surveillance and management. This can help shorten outbreak duration and support identification of the causative agent(s) so that appropriate corrective measures can be designed and implemented. Molecular methods such as polymerase chain reaction (PCR) and next-generation sequencing (NGS) allow multiple pathogens to be genomically profiled at the same time, and, in some cases, without a dependency on isolate culture. This effectively reduces the time and testing needed to identify pathogens present in samples collected from an outbreak. In this study, we demonstrate proof of concept and describe a microfluidics-based protocol designed to detect and identify four upper respiratory pathogens in up to 48 samples from a single run using an automated workflow starting with nucleic acid derived from saliva (extraction free). The use of nanoliter-scale microfluidic reactions conserves precious reagents while reducing plastic waste and enabling sustainable lab operations. Following preparation of sample and assay mixes, which are dispensed into an integrated fluidic circuit (IFC) that is subsequently loaded onto the Biomark™ X9 System for High-Throughput Genomics for processing, results are available in two hours without manual intervention. The current list of targeted pathogens includes influenza A, influenza B, respiratory syncytial virus and SARS-CoV-2, and can be further expanded and customized using the open architecture of the IFC. Each of the IFC"s 48 assay inlets connects with an independent reaction chamber, which enables all assays to share a common fluorophore and thermal profile while preventing assay-to-assay interference associated with multiplex reactions.

**Methods:** Well-characterized, commercially available samples for the viral targets [NATtrol™ SARS-CoV-2 External Run Control (PN NATSARS(COV2)-ERC) and Flu Verification Panel (PN NATFVP-NNS)] were purchased from ZeptoMetrix®. Equal volumes of control material and donor saliva were mixed together and RNAsecure™ RNase Inactivation Reagent (Thermo Fisher Scientific™, PN AM7005) was added to the saliva-control mixtures to a final concentration of 1X. The mixtures were subjected to heat denaturation at 90 °C for 10 minutes to extract the viral RNA. A targeted one-step reverse transcription/preamplification was performed on a standard thermocycler using Standard BioTools™ Advanta™ RT PA MM (RT-preamplification master mix), and the cDNA was diluted 1:5 in DNA Suspension Buffer (Teknova, PN T0227). The diluted cDNA was mixed with Advanta PCR MM and sample loading reagent and loaded into the sample inlets of a Standard BioTools 48.48 Dynamic Array™ IFC-X Real-Time PCR.

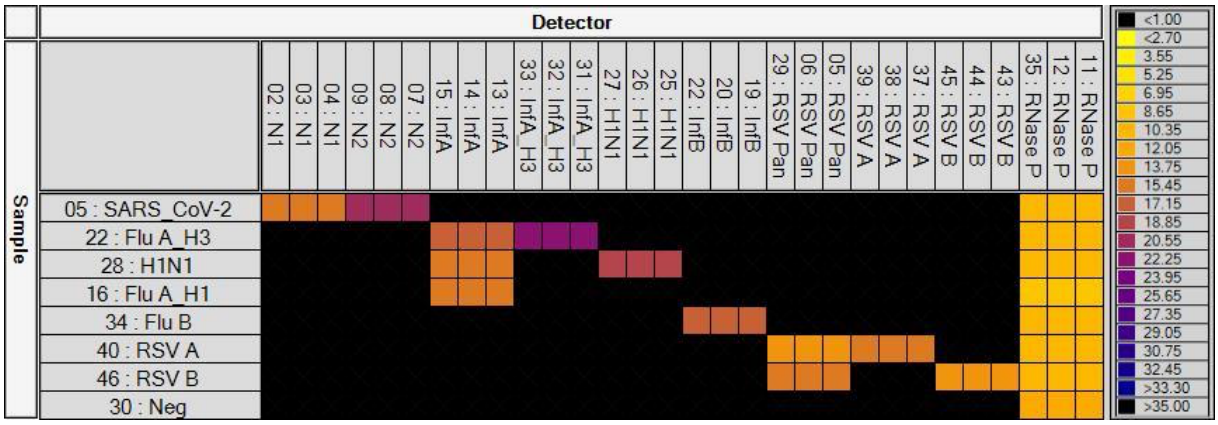
Individual probe-based assays for the viral targets and RNase P as an internal control were mixed with assay loading reagent and added to the assay inlets of the 48.48 IFC. The IFC was placed in a Biomark X9™ System for sample-assay mixing, cycling and data capture. Data was analyzed using Standard BioTools Real-Time PCR Analysis Software followed by a custom interpretative script. allowing for flexibility in the assays an end user wishes to test as well as the ability to quickly add or subtract assays on a per needed basis. The ability to perform replicate assays within the same IFC reduces variability and gives greater confidence in the data collected before reporting. Further studies for this panel will include using extracted viral genome, the addition of more targets and subtyping

assays, and a more detailed interpretive software that will further report the subtype for pathogens that require it.

**Results:** Successful amplification and identification of viral targets were achieved using the 48.48 IFC on the Biomark X9 System. Heat map data depicts the reaction chambers that amplified viral targets using cycle threshold (Ct) values, which are color-coded (Figure 2). Additionally, the heat map data shows specificity of the assays as indicated by no cross-reactivity with the other pathogens analyzed in the panel. In instances in which an additional subtype assay was used, the subtype was positively identified and did not cross-react with other subtypes within the same species. For example, a swine flu (H1N1) assay was designed to specifically react with H1N1 and not other H1 subtypes. The heat map data and amplification curves (Figure 3) show that for influenza A H1, no cross-reactivity is observed with the H1N1 assay. One of the features of the Standard BioTools Real-Time PCR Analysis Software is the ability to write custom interpretive scripts to generate a report that identifies which pathogen is detected in a sample (Figure 4). The use of replicate assays loaded in the IFC is leveraged in the interpretive software to give greater confidence in the call of Detected vs. Not Detected output in the interpretive report.

**Conclusions:** In this study, we show the ability to quickly screen for the most common respiratory pathogens in a single run. The power of Standard BioTools microfluidic technology is in its ability to quickly test any sample for a multitude of targets in singleplex and in one fluorescent channel. This can be done with just a few microliters of sample and master mix, cutting down on cost. Since the IFC digitizes the samples and assays into individual reaction chambers, assay design is simplified, and cross-reactivity is less of a concern than in multiplex detection systems. The IFCs are open, allowing for flexibility in the assays an end user wishes to test as well as the ability to quickly add or subtract assays on a per needed basis. The ability to perform replicate assays within the same IFC reduces variability and gives greater confidence in the data collected before reporting. Further studies for this panel will include using extracted viral genome, the addition of more targets and subtyping assays, and a more detailed interpretive software that will further report the subtype for pathogens that require it.

**Figure**




## P-011

# Wastewater surveillance of antimicrobial resistance and wastewater treatment efficiency in Mbarara Uganda and Karlsruhe, Germany

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**Background and objectives:** On the one hand, wastewater-based surveillance of antimicrobial resistance (AMR) has emerged as an alternative to traditional clinical surveillance, as it can provide information on the entire population served by the sewerage system [1]. On the other hand, it is important to determine the removal efficiency of the existing wastewater treatment system in order to assess the contamination released into the environment and the risks to human and animal health [2].

**Methods:** We compared the occurrence and fate of indicator bacteria, antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) between a pond system in Mbarara, Uganda and an advanced wastewater treatment plant (biological treatment and activated carbon filtration) in Karlsruhe Germany. Furthermore, three German wastewater treatment plants using ozonation as an advanced treatment step were also investigated. Extended-spectrum beta-lactamase (ESBL)-producing *E. coli* were detected using CHROMagar™ ESBL medium followed by MALDI-TOF-MS identification. Monitoring of key ARGs was performed at by quantitative PCR analysis.

**Results:** The results of this study showed that in Uganda 30.6% of the total *E. coli* in raw wastewater were ESBL producers, a high proportion compared to Germany (1.1 %) and other countries [3]. Our study also revealed the widespread presence of the carbapenemase gene $bla_{\text{CMY-2}}$ in the Mbarara population, while the gene $bla_{\text{OXA-48}}$ was detected more prevalent and in higher concentrations in Karlsruhe. With regard to the elimination through wastewater treatment, the stabilisation pond system in Uganda showed only a minor reduction in total and ESBL *E. coli* (2.0-2.4 log levels) and an increase in ARGs (*sul1*, *tet*C and $bla_{\text{CMY-2}}$, see Figure). In Germany, a reduction of 4.3 to 4.6 log levels for total and ESBL *E. coli* and 1.6-2.7 log levels for ARGs was measured by advanced wastewater treatment. The use of ozonation as an advanced treatment step (ozone doses of approx. 3 mg/L) resulted in a further 1-2 log reduction in bacterial parameters.

**Conclusions:** The results suggest that: i) wastewater-based monitoring can serve as a cost-effective method for understanding and mitigating public health threats, especially in low- and middle-income countries; ii) wastewater treatment is an important barrier to the entry of AMR into the environment, with advanced treatment systems achieving significantly higher reductions than pond systems; and iii) the use of ozone can be recommended when expanding wastewater treatment plants.

**Acknowledgements:** This study was financially supported by the German Federal Ministry of Education and Research (BMBF 02WIL165) as part of of the German-Israeli Cooperation in Water Technology Research and the ERA-NET Cofund AquaticPollutants (grant agreement number 869178-AquaticPollutants) – SARA project (BMBF grant number 02WAP1618).

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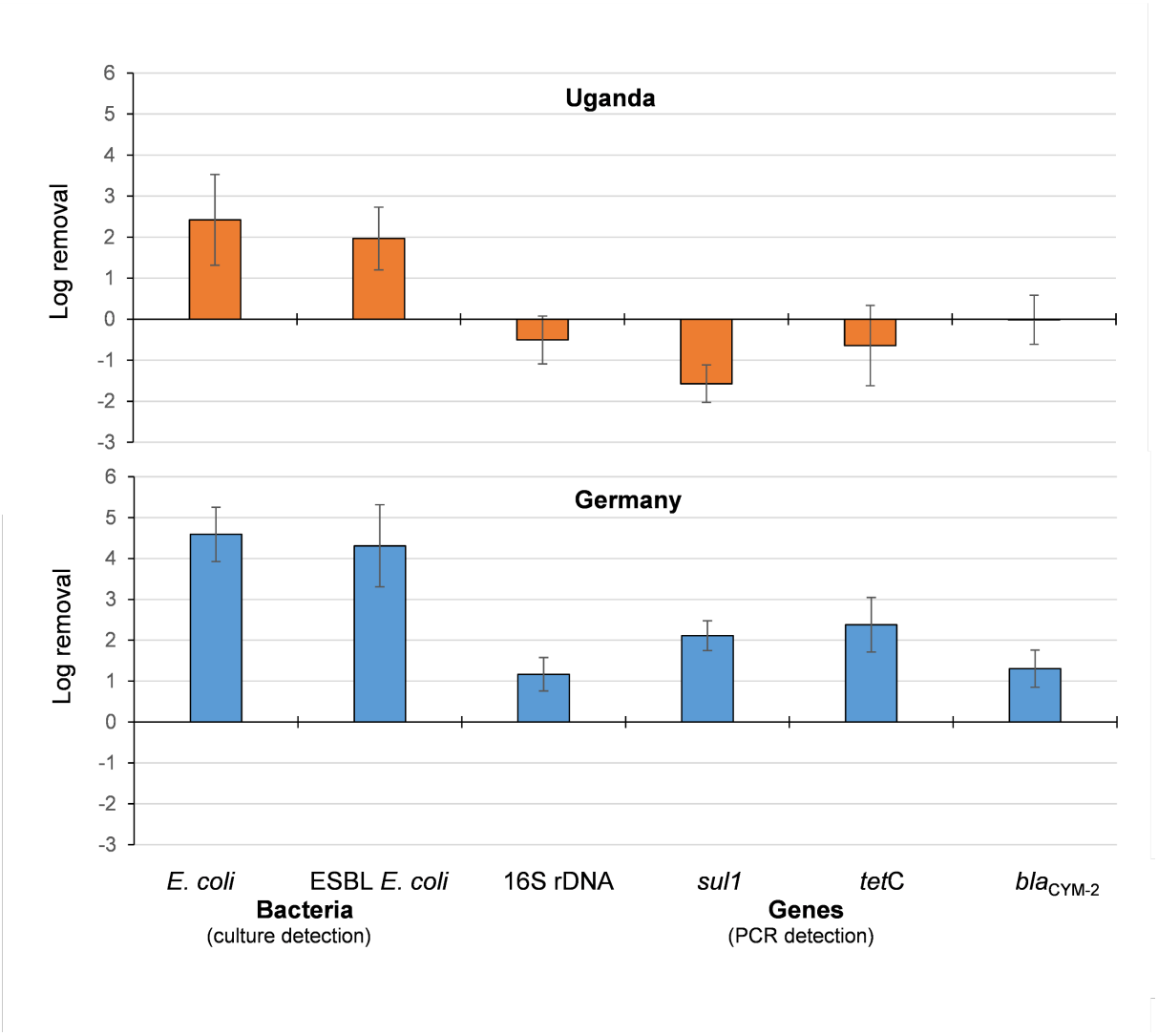
**Figure**




## P-012

# Prevalence of Neomycin Resistance *E. coli* in Dutch Livestock in 2024

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**Background and objectives:** Neomycin (NEO) is a first-choice antibiotic approved for treatment of colibacillosis in cattle, pigs, sheep, goats and poultry. The recent increase of neomycin resistance (NEO-R) in *E. coli* reported from Denmark (in pigs) and France (in cattle) is concerning. For this reason a CoVetLab project called NEO-TRACK was conducted to determine the prevalence and characterisation of NEO-R *E. coli* in livestock in five European countries (Denmark, Sweden, France, UK and the Netherlands) mainly focussing on the presence of the *aph(3")-Ia* gene. Here we describe the results of a prospective screening of Dutch caecal samples from pigs, veal calves and broilers and feacal samples from dairy cattle.

**Methods:** In 2024, over a hundred caecal samples per animal species from the Dutch monitoring program for antimicrobial resistance in livestock [3] were cultured on MacConkey agar with 16 mg/L NEO for the isolation of NEO-resistant *E. coli*. NEO-R resistance mechanisms were detected by whole genome sequencing.

**Results:** In 2024, the prevalence of NEO-R *E. coli* in Dutch veal calves was 62% (n=74 out of 120 samples), in broilers 58% (n=63 out of 108 samples), in pigs 43% (n=124 out of 124 samples), and in dairy cattle 1% (n=1 out of 113 samples).

WGS analysis of the NEO-R *E. coli* showed *aph(3')-Ia* in 90% of the isolates. Related *E. coli* were found in multiple livestock sectors, for example ST10 was detected in isolates from broilers, veal calves and pigs; ST88 was detected in pigs, dairy cattle and veal calves; ST58, ST1433 and ST206 were detected in pigs and veal calves.

**Conclusions:** This study shows that *aph(3")-Ia* plays a major role in neomycin resistance in indicator *E. coli* in Dutch livestock and is widely spread across multiple ST types. These results confirm the further spread of this NEO-R gene amongst *E. coli* in livestock.

## P-014

# Antibiotic-Resistant Bacteria in Wild Raptors Across the Palearctic Realm

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**Background and objectives:** Wild birds are recognized as carriers and reservoirs of antibiotic-resistant bacteria (ARB) and play a significant role in the environmental dissemination of antimicrobial resistance. The study aimed to assess the prevalence and genetic determinants of Enterobacterales resistant to clinically important antibiotics in wild raptors.

**Methods:** Between 2018 and 2022, a total of 863 cloacal swabs were collected from raptors, primarily Red Kites (*Milvus milvus*) and Black Kites (*Milvus migrans*) (n=756), across the Palearctic Realm: Czech Republic (n=470), Germany (n=116), Russia (n=133), Austria (n=52), Spain (n=51), Slovakia

(n=17), Ukraine (n=13), and Hungary (n=11). Sampling sites were classified as remote, agricultural, urban, or mixed, allowing an analysis of bacterial colonization in raptors across different environments. Cultivation was performed on media with cefotaxime, ciprofloxacin, colistin, and meropenem to isolate resistant Enterobacterales. Isolates were screened for clinically relevant antibiotic resistance genes (ARGs) by PCR and subjected to whole-genome sequencing, followed by bioinformatic analysis, with focus on ARGs and virulence genes.

**Results:** We obtained (25.7%; n=222/863) isolates carrying ARGs; n=172 of them identified as *Escherichia coli*, while n=50 belonged to other genera. The majority (77%; n=170/222) of isolates exhibited multidrug resistance. Genes encoding extended-spectrum and AmpC β-lactamases (n=146), along with plasmid-mediated quinolone resistance genes (n=103) were detected. Furthermore, transmissible resistance to colistin (n=11) and meropenem (n=1) was found. ARGs were more common in human-impacted regions, whereas virulence genes were associated with remote areas. *E. coli* isolates showed high phylogenetic diversity, represented by 100 sequence types, with ST10 being the most frequent (8%).

**Conclusions:** High prevalence of clinically important bacteria with transferable resistance in Kites, especially from human-impacted environments, raises concerns about the environmental spread of resistant pathogens. Presence of ARB in these raptors may be linked to their feeding strategy. Kites, as opportunistic scavengers, frequently forage at municipal landfills, where they are exposed to waste products of both animal and human origin. Such environments pose a significant risk for the acquisition and dissemination of ARB, including strains resistant to clinically relevant and last-resort antibiotics.

## P-015

# Molecular investigation of *Enterococcus faecalis* and *Enterococcus faecium* from diseased animals collected within the GE*RM*-Vet resistance monitoring

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**Background and objectives:** The occurrence of infectious diseases is a serious threat in the veterinary health sector. In this context and to maintain animal health it is important to gain knowledge on the antimicrobial resistances in bacterial pathogens in order to identify effective treatment options. *E. faecalis* and *E. faecium* causing infections in animals as mastitis in cattle, sepsis or urogenital infections in poultry or small animals were monitored with the German antibiotic resistance monitoring of veterinary pathogenic bacteria.

**Methods:** Within the GE*RM*-Vet national monitoring at the Federal Office of Consumer Protection and Food Safety (BVL) *Enterococcus* spp. isolates (n=169) were collected from cattle, companion animals and poultry in 2023. Antimicrobial susceptibility testing against 24 substances was performed via broth microdilution method according to CLSI VET01S-standard. Whole-genome sequencing on Illumina MiSeq (DNA-library preparation with Illumina DNA prep kit, MiSeq V3 2x300, AQUAMIS QC and *de novo*-assembly, followed by BakCharak analysis) was used for molecular resistance analysis and determination of sequence types (ST) of *E. faecalis* und *E. faecium*.

**Results:** In isolates causing mastitis in dairy cattle erythromycin resistances were observed in 4% (*E. faecalis*) and 28% (*E. faecium*) of isolates. Tetracycline MIC90 were at 128mg/L (*E. faecalis)* or 32mg/L (*E. faecium)* whereas gentamicin MIC90 at 16mg/L (*E. faecalis)* or 8 mg/L (*E. faecium).* Among *E. faecalis* from poultry, 27% of isolates were shown to be erythromycin resistant and MIC90 of 16mg/L for gentamicin and 128mg/L for tetracycline were measured. 15% of *E. faecalis* from small animals were erythromycin resistant, 47% of isolates were tetracycline resistant. Their gentamicin

MIC90 was detected to be at 16mg/L. Among small animals and poultry (one isolate each) high-level-gentamicin resistance (MIC=>256 mg/L) was caused by the *aac*(6')-Ie/*aph*(2")-Ia gene; isolates belonged to ST16 (cat) and ST314 (poultry). Among all enterococcal isolates with tetracycline MIC of 32-128mg/L, mainly the *tet*(M) gene was seen; *tet*(L)-*tet*(M) were responsible for high tetracycline MIC in the majority of poultry isolates. The genes *erm*(A) und *erm*(B) were detected in *Enterococcus* with erythromycin MIC of >32mg/L, whereas *erm*(A) was exclusively seen in poultry isolates. Resistances towards linezolid or vancomycin were not observed.

**Conclusions:** Since enterococcal pathogens and their antibiotic resistances also play a role in human medicine, veterinary surveillance of *E. faecalis* and *E. faecium* causing infections including whole genome sequencing provides an important contribution to detect resistant pathogens.

## P-016

# Genetic characterization of resistant *Escherichia coli* isolated from imported tortoises that died during quarantine

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**Background and objectives:** Antimicrobial resistance (AMR) is recognized a major global public health threat. The international trade of pet animals contribute significantly to the dissemination of AMR bacteria, as animals may carry resistant pathogens without exhibiting symptoms, thereby acting as reservoirs and vectors [1,2]. This study aimed in genome characterisation focused on the identification of antimicrobial resistance genes in *Escherichia coli* isolated from tortoises imported to Poland that died during quarantine.

**Methods:** Thirty-three *E. coli* were obtained from sixteen tortoises, as described in our previous study [1]. The isolates were tested for minimal inhibitory concentration (MIC) determination with a microbroth dilution method using Sensititre EUVSEC3 plates and – if cephalosporin resistance was observed - with Sensititre EUVSEC2 panels (Trek D.S.). The results were interpreted according to the criteria listed in the Commission Implementing Decision (EU) 2020/1729. Whole genome sequencing (WGS) was performed on the NextSeq Illumina platform. The *staramr* tool (version 0.9.1, Galaxy Aries platform) was applied for *in silico* analyses.

**Results:** All strains were resistant to at least one antimicrobial. Notably, all exhibited resistance to ciprofloxacin often accompanied by the resistance to nalidixic acid, tetracycline, ampicillin, trimethoprim cefotaxime, ceftazidime, and chloramphenicol (79%, 76%, 67%, 61%, 52%, 40%, and 18%, respectively). The isolates displayed high sequence type (ST) diversity (n=15), with ST351 (n=5), ST6743 (n=5), ST162 (n=4), and ST410 (n=4) being the most prevalent. Ciprofloxacin resistance was determined by the presence of *qnrS1* and *qepA4* genes, as well as mutations in *gyrA* (D87H), *gyrA* (D87N), *gyrA* (S83L), *parC* (S80I), *parE* (S458A), *parC* (E84G).Cephalosporin resistance was associated with the presence of *blaCTX-M-15* genes. Ampicillin resistance was encoded by *blaOXA*-1, blaTEM-*214*, *and blaTEM-1B* genes. Resistance to chloramphenicol, tetracycline, and trimethoprim resistance were determined by i.e.: *catB3*, *catA1*, *cmlA, tet(A), tet(B), dfrA14*, *dfrA17*, and *dfrA12* genes.

**Conclusions:** The confirmation of resistance to various types of antimicrobials, including substances of the highest priority for human medicine, e.g., cephalosporins and quinolones, in a variable *E. coli* from imported turtles confirmed that these animals constitute a significant reservoir of multidrug-resistant bacteria. The presence of multidrug-resistant *E. coli* was possibly a result of industrial

breeding practices and the extensive use of antimicrobials. These hazards indicate the need for a systematic survey of exotic pets.

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## P-017

# Towards a national antimicrobial resistance surveillance system in food-producing animals in Senegal

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**Background and objectives:** One of the main pillars of the FAO Action Plan on AMR 2021–2025 [1] is to strengthen surveillance and research. In Senegal, a functional AMR surveillance system in animals yet has to be established. However, recent ad-hoc studies, such as the 2022 Fleming Fund (phase I) project on *E. coli* and *Salmonella* spp. resistance in poultry, and the 2024 PRAPS project (i.e. Regional project to support pastoralism in Sahel) on *E. coli, Salmonella* spp. and *Staphylococcus* spp. resistance in ruminants have showed concerning resistance levels to various antibiotics of importance to human and animal health. In addition to these ad-hoc studies, the National Livestock and Veterinary Research Laboratory (LNERV) performs routine diagnostic activities, including antimicrobial susceptibility testing of bacterial pathogens isolated from food-producing animals, especially from intensive or semi-intensive farming (approx. 300 isolates each year). Building on these existing AMR data centralized at LNERV, and with support from the Fleming Fund (phase II), the Senegalese veterinary authorities aim to initiate a national AMR surveillance system in food-producing animals. This paper describes the strategy envisaged to develop such a system within the next two to five years.

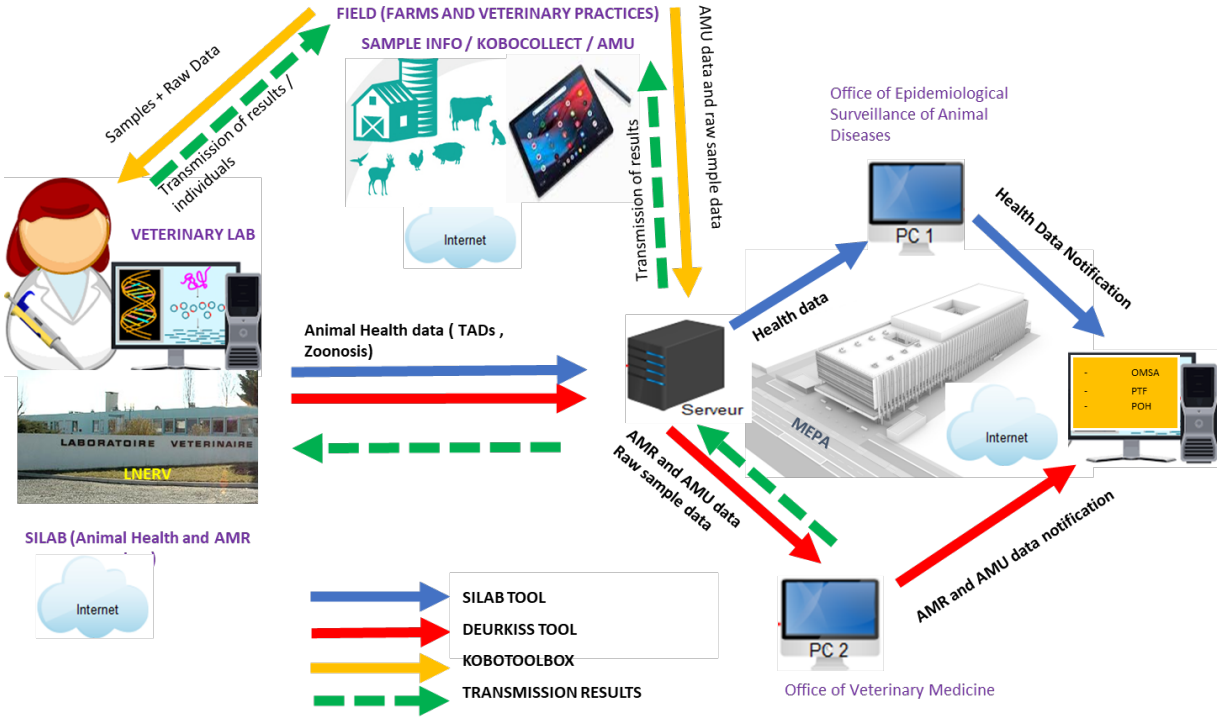
**Methods:** The national AMR surveillance system will consist of three main components closely related together (Figure 1), namely i) a National Coordination Centre (NCC) located at the Division of Veterinary Medicine and Pharmacy of the national veterinary services, which will be in charge of managing, analyzing and reporting the data originating from the LNERV; this will be carried out using the FAO InFARM system [2], ii) a National Reference Laboratory, in this case the bacteriology laboratory of LNERV, which will perform antimicrobial susceptibility tests using the disk diffusion method on isolates collected via routine diagnostic (passive surveillance) and ad-hoc studies (active surveillance); generated data will be submitted electronically to the NCC via their LIMS. LNERV also

has a molecular biology platform (PCR, WGS by short-reads and long-reads), designated as a SeqAfrica ONT sentinel site, and will be able to characterize AMR molecular mechanisms, hence facilitating the interpretation of AMR phenotypic data, and iii) selected AMR sentinel sites linked to veterinary clinics located in different country areas, that will help with collection of samples and associated metadata as well as transport of material to the LNERV. Additional local laboratories might be included at a later stage depending on available resources to build capacity for antimicrobial susceptibility testing.

**Results:** Expected results include proportions of resistances to antibiotics among the major commensal (*E. coli*, *Enterococcus faecium* and *faecalis*), zoonotic (*Salmonella* spp.*)* and pathogenic (*Staphylococcus* spp., *Pasteurella* spp., *Enterococcus* spp., *Streptococcus* spp. and *Klebsiella* spp.) bacteria from food-producing animals in Senegal. These data will be used to inform AMR risk assessment and risk management at the national level. In addition, a contribution to international monitoring of AMR in food-producing animals is expected via the participation of Senegal to the FAO InFARM surveillance system [2].

**Conclusions:** An initiative has been launched to develop an AMR surveillance system in food-producing animals in Senegal, building on existing data and capacity. This initiative should contribute to close a gap on AMR surveillance in animals in LMICs, and inspire other countries willing to progress towards improved AMR control in the animal sector.

**Figure**



**P-018**

# Antimicrobial resistance in *Salmonella* Infantis isolated from poultry and swine (2021–2024)

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**Background and objectives:** Salmonellosis is the second most common foodborne zoonosis in the EU. Attention towards the *S.* Infantis serovar (antigenic formula 6,7,14:r:1,5) has significantly increased in recent years due to the rising occurrence and resistance, including (multidrug resistance) (MDR) [1]. Although Salmonella control programs (NSCP) are implemented in the poultry sector, pig production is neglected — moreover, the control measures in poultry target few serovars, and *S.* Infantis is included just in breeding flocks of *Gallus gallus*.

**Methods:** NSCPs implemented in Poland require *Salmonella* isolates submission to a reference laboratory. NVRI runs national *Salmonella* monitoring of swine herds rather than controlling programs. Both sources (poultry and swine) during 2021-2024 resulted in the identification of 584 *S.* Infantis isolates. They were tested according to EN ISO 6579-1:2017+A1:2020-09 standard and identified according to the White–Kauffmann–Le Minor scheme using slide agglutination tests with commercial antisera. A subset of 130 isolates was tested for antimicrobial susceptibility with the microbroth dilution method (Sensititre EUVSEC3 plate; TREK Diagnostic Systems). The results were interpreted according to the criteria set in the Commission Implementing Decision (EU) 2020/1729 . Whole Genome Sequencing (WGS) was performed on X strains with the NextSeq platform (2x300bp; Illumina). Tools included on the Staramr (Galaxy Aries) were applied for analysis of the genetic content of sequences.

**Results:** Over 69,2% (n=90) of strains were resistant to at least one antimicrobial . Of all antimicrobials assessed ciprofloxacin, nalidixic acid and tetracycline resistance dominated (98,9%, 97,8%, and 80,0% respectively). A lower percentage of resistance was observed for ampicillin (34,4%), trimethoprim (8,9%), chloramphenicol (6,7%) and gentamicin (2,2%). One strain was resistant to amikacin (1,1%). No cephalosporin or carbapenem-resistant isolates were noted. WGS revealed that all *S.* Infantis belonged to ST32 and were characterized by numerous resistance determinants. Resistance to ciprofloxacin was determined by the presence of *qnrS1* gene and mutations in *gyrA*(S83Y), *gyrA* (D87G). Phenotypic resistance resulted from the presence of the *dfr*A14 for trimethoprim and *bla*TEM-1B and *bla*TEM-220 genes for ampicillin, respectively. The *IncFIB*(pN55391) plasmid replicon was the most frequently identified.

**Conclusions:** Our study indicates that *S.* Infantis showed resistance against antimicrobials which are commonly used in human and veterinary medicine as first-line treatment [2]. Multidrug resistance may be caused by excessive use of antimicrobials or clonal spread of bacteria. Further monitoring of antimicrobial resistance in this serovar is necessary along the entire food chain.

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**P-019**

# Analysis of *Staphylococcus aureus* CC1 and CC1660 from humans and horses

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**Background and objectives:** Human and equine *Staphylococcus aureus* isolates of the clonal complexes (CCs) CC1 and CC1660 were investigated for their genetic relationships, the presence of virulence genes and their antimicrobial resistance properties.

**Methods:** Ninety-three *S. aureus* isolates (64 human, 29 equine) were investigated by whole-genome sequencing (WGS) with Illumina MiSeq and sequence analysis by multilocus sequence typing (MLST), core genome MLST (cgMLST) and *spa* typing as well as screened for antimicrobial resistance and virulence genes. Antimicrobial susceptibility testing was performed for 31 antimicrobial agents [1,2].

**Results:** The WGS confirmed 77 CC1 and 16 CC1660 isolates. MLST detected 13 sequence types (STs) in CC1 and four in CC1660. The minimum difference between the two CCs was 1331/1430 alleles according to cgMLST. The difference between the isolates ranged from 0 to 269 in CC1 and from 0 to 233 alleles in CC1660. The isolates of CC1 and CC1660 belonged to 10 and five different *spa* types, respectively. Antimicrobial susceptibility testing identified penicillin resistance via *blaZ* in 74 isolates. Ten of these were also oxacillin-resistant; with seven positive for *mec* genes. Resistance to gentamicin (*aacA-aphD*), tetracycline (*tet*(L)/*tet*(K)) and neomycin (minimum inhibitory concentration (MIC) ≥4, *aadD* or *aphA3*) was seen in 33, 32 and 32 isolates, respectively. All isolates were susceptible to vancomycin, linezolid and quinupristin-dalfopristin. The equine leucocidin genes *lukP/Q* were found in 24 isolates of equine origin and 38 isolates from humans, of which 35 had confirmed contact to horses. No Panton-Valentine leucocidin genes *lukF-PV* and *lukS-PV* were found. Three human CC1660 isolates carried the toxic shock syndrome toxin-1 gene *tst.*

**Conclusions:** The investigation of the 93 isolates by cgMLST revealed two clearly separated CCs, CC1 and CC1660. Antimicrobial susceptibility testing revealed various resistance patterns, that should be monitored in the future.

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**P-020**

# Biocide susceptibility in *Staphylococcus aureus* CC1 and CC1660

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**Background and objectives:** A collection of human and equine *Staphylococcus aureus* isolates of the clonal complexes (CCs) CC1 and CC1660 was investigated for their phenotypic biocide susceptibility, the presence of biocide resistance genes and their efflux capacity.

**Methods:** The collection of 93 *S. aureus* isolates (55 human CC1, 9 human CC1660, 22 equine CC1 and 7 equine CC1660) was subjected to broth microdilution assays for benzalkonium chloride (BAC), octenidine (OCT), polyhexanide (PHX) and chlorhexidine (CHX) [1]. All isolates were investigated for the presence of *qac* genes. In 26 isolates *qac* genes were confirmed through PCR. The presence of increased efflux activity was screened indirectly by evaluation of the fluorescence emitted by ethidium bromide (EtBr) accumulated inside the cells, through (i) cartwheel assays [2], performed for the 26 *qac* positive isolates and one control isolate without a *qac* gene; and (ii) real time fluorometry [3], performed for eight of these isolates. Two control strains [4] were included in both these assays.

**Results:** Biocide susceptibility testing revealed unimodal distributions for all tested biocides. For BAC, minimum inhibitory concentrations (MICs) of 0.000125-0.0005% were detected. The *qac* genes were found in all 24 isolates with a BAC MIC of 0.0005% (19 *qacA*, 4 *qacC*, 1 *qacC* S99L) and two (*qacC*) of the 19 isolates with a MIC of 0.00025%. The cartwheel assays confirmed increased efflux activity in all but one *qacA*-positive isolates and all *qacC*-positive isolates. This efflux activity was confirmed in real time fluorometric assays for the selected isolates. The isolate carrying the *qacC* mutation S99L showed a lower level of efflux activity by both methods when compared to the other *qacC*-positive isolates.

**Conclusions:** The efflux activity of *qac*-positive *S. aureus* isolates can decrease their susceptibility to BAC. To further investigate this effect, susceptibility testing for BAC with efflux inhibitors will be performed.

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**P-021**

# Investigation of the Prevalence and Antimicrobial Resistance of Coagulase-Negative Staphylococci in Bovine Mastitis in Selected Regions of China

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**Background and objectives:** Bovine mastitis is a prevalent disease in dairy herds, leading to huge economic losses and posing risks to food safety and public health as well as animal welfare. The prevalence of coagulase-negative *staphylococci* (CNS) in mastitis cases has risen in recent years [1,2]. However, comprehensive data on CNS species distribution and antimicrobial resistance in bovine mastitis remains scarce.This study investigates the prevalence and antimicrobial resistance of CNS isolates from bovine mastitis in three different areas of China, aiming to provide evidence for preventing and controlling bovine mastitis.

**Methods:** A one-year cohort study was conducted at three dairy farms in three cities (Hohhot, Daqing, and Nanjing). A total of 463 clinical mastitis milk samples were collected in September 2022, March 2023, June 2023, and September 2023. Bacterial isolation was performed according to NMC guideline and antimicrobial susceptibility was assessed using the broth microdilution method.

**Results:** From 463 samples, 535 bacterial pathogens were isolated, including 140 CNS strains (26.17%). The CNS isolates including *Staphylococcus sciuri* (n=43, 30.71%), *S. auricularis* (n=33, 23.57%), *S. chromogenes* (n=19, 13.57%), *S. simulans* (n=12, 8.57%), *S. equorum* (n=8, 5.71%), *S. xylosus* (n=7, 5.00%), and *S. lentus* (n=4, 2.86%). Distribution of CNS was regional and seasonal specific, with the highest prevalence in Daqing and Nanjing during summer, and in Hohhot during autumn. *S. sciuri* was most prevalent in Daqing across all seasons, while Hohhot demonstrated predominance of *S. auricularis* in autumn and *S. sciuri* in spring. *S. chromogenes* was the dominant species in Nanjing. Antimicrobial susceptibility testing revealed high resistance rates to lincomycin (85.07%), oxacillin(62.69%), penicillin (44.78%), and ampicillin (38.81%). Multi-drug resistance was prevalent, with 35.82% of isolates exhibited resistance to ≥5 antimicrobials, with the maximum resistance observed against 12 distinct antimicrobials. Isolates were sensitive to ceftiofur (22.39%), cephalexin (23.88%), and gentamicin (2.99%). *S. sciuri* exhibited 100% resistance to lincomycin and 52.94% to ciprofloxacin; *S. auricularis* showed resistance to lincomycin (73.33%), penicillin (66.67%), and ampicillin (66.67%); while *S. chromogenes* demonstrated resistance primarily to lincomycin (84.62%).

**Conclusions:** CNS emerged as the predominant clinical pathogen with a high multidrug resistance rate in Chinese dairy herds. Ceftiofur, cefalexin, and gentamicin may be effective alternative antimicrobials to treat CNS mastitis whereas lincomycin is not suitable. Tailor-made antimicrobial therapy for bovine mastitis caused by CNS should account for epidemiology of local CNS to improve treatment success and curb the spread of resistant strains.

**P-022**

# Dynamics of antimicrobial resistance genes in weaned pigs: determining abundances and animal individual differences by qPCR

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**Background and objectives:** The frequency of antimicrobial drug administration is higher in young pigs, particularly piglets and weaners than in older groups (Rennings *et al*., 2015; Schaekel *et al*., 2017). This may cause higher antimicrobial resistance (ARGs) in weaners compared to fattening pig as indicated in Petrin *et al*. (2019). In this study, we monitored ARGs abundances in weaner pig to assess inter-individual differences and temporal trends in individuals and herds.

**Methods:** A batch of weaners at the age of 4 weeks received from one breeder were housed in three identical pens in a pre-cleaned flat deck for eight weeks. Fecal samples from individual pigs (n = 103) and pooled pens floor herd feces (n = 53) were investigated for eight antimicrobial resistance genes (*aadA1*, *blaTEM*, *dfrA12*, *ermB*, *lnuF*, *qnrS*, *sul2*, *tet(A)*) using real-time qPCR.

**Results:** All samples contained ARGs. Notably, *ermB* and *aadA1* were the most prevalent, while *dfrA12* and *qnrS* were the least abundant. There was quite high interindividual variation in gene copies among the examined pigs for all ARGs, except for *ermB*. The pooled pen floor fecal samples exhibited significantly higher loads of ARGs compared to individual samples. The analysis of individual temporal dynamics over an eight-week period showed that the average number of *16S rRNA* gene copies was significantly lower in the first half compared to the second half. In contrast, the gene copies of *sul2* and *tet(A)* were found to be significantly lower in the second half.

**Conclusions:** The study showed high variability in ARGs abundances between individual pigs, even though they were reared under the same conditions. Surveillance of ARGs in the barn is more effective using pooled fecal samples than samples from individual animals in weaner pigs. Temporal dynamics over eight weeks revealed that animal individual factors influenced ARG levels.

**P-023**

## Antibiotic resistance and first comprehensive genomic characteristics of *Salmonella* Gallinarum strains isolated in Morocco

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**Background and objectives:** Fowl typhoid, caused by *Salmonella enterica* subspecies enterica serovar Gallinarum, is among the most important diseases listed by World Organization for Animal Health (WOAH) and have resulted in considerable economic losses to poultry industry over the world.

**Methods:** In this study, 23 *Salmonella enterica* subspecies enterica serovar Gallinarum biovars Gallinarum were isolated and identified from poultry in Morocco showing typical lesions of fowl typhoid, including splenomegaly and hepatomegaly with necrotic foci and peritonitis. The isolates were investigated for antibiotic resistance, virulence and genetic diversity. The isolates were tested for phenotypic antibiotic susceptibility. The Whole Genome Sequencing (WGS) was used for studying the genomic characteristics of isolated strains and to determine the antibiotic resistant markers.

**Results:** Antibiotic susceptibility testing revealed that 13 (56.5%), 8 (34.8%) and 9 (39.1%) isolates were resistant to quinolones, tetracycline and colistin, respectively. WGS revealed 26 resistance genes, coding mainly for efflux pumps and beta-lactamase, including *aac*(6")-Iaa, *aac*(3)-IIa and *bla*TEM-1a. Virulence profiling identified 107 genes and multiple Salmonella Pathogenicity Islands (SPIs), while mobile genetic elements analysis detected the IncI1-(α) plasmid in two strains, previously unreported in avian SG. Phylogenomic analysis showed that, the isolated strains in this study were closely related to SG strains in the NCBI database, with 8 strains phylogenetically linked to the SG9R vaccinal strain. Single Nucleotide Polymorphism (SNP) analysis, using SG9R as a reference, revealed a low number of variants in these 8 strains, with SNPs in *rfa*J and *ace*E genes—markers of attenuation—suggesting reversion to virulence.

**Conclusions:** These findings of this study enhance understanding of SG"s genomic features, aiding improved management of FT, vaccine program development and reduction of antibiotic resistance in poultry populations.

**P-024**

## Identification of the antimicrobial resistance profile of *Escherichia coli* isolated from migratory wild birds *Milvus migrans*

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**Background and objectives:** Antimicrobial resistance (AMR) is one of the most pressing threats to global health. Identifying the reservoirs and vectors of AMR is therefore essential. Wild birds, including raptors, can serve as potential reservoirs and disseminators of antibiotic-resistant bacteria through local, intercontinental, and intracontinental migration. This study aimed to determine the phenotypic

and genotypic antibiotic resistance as well as the virulence associated genes of 16 *Escherichia* (*E.*) *coli* isolated from cloacal samples of 15 Black Kites (*Milvus migrans*) captured at the Jabal Musa Balyounish Reserve in Morocco before crossing the Strait of Gibraltar.

**Methods:** Phenotypic antimicrobial resistance of *E. coli* against 13 antimicrobials was assessed by using the Kirby-Bauer Disk Diffusion Susceptibility Test. Resistance to colistin was evaluated by minimum inhibitory concentration (MIC) testing and confirmed using ColiSpot. Whole genome sequencing (WGS) of all isolates was performed using the Illumina NovaSeq 6000 platform. In-silico pipeline was used to determine serotypes, sequence types and phylogenetic groups using ECTyper, MLST and EZClermont, respectively. Genotypic resistance was annotated using various databases in Abricate.

**Results:** The results showed a strong correlation between phenotypic and genotypic resistance profiles. Most isolates (62.5%) belonged to the B1 phylogenetic group and exhibited 21 different O-types, 12 H-types, and 10 sequence types (STs), including ST648 and ST155, which are commonly associated with antibiotic-resistant and virulent *E. coli* strains. Moreover, 50% of isolates displayed multidrug resistance, with ampicillin (10/16), fosfomycin (9/16) and tetracycline (8/16) being the most prevalent resistances. Genomic analysis identified the corresponding resistance genes, including *mcr*-1 in the only phenotypic colistin-resistant isolate.

**Conclusions:** These findings suggest that migratory birds play a significant role in the dissemination of multidrug resistant bacteria, emphasizing the need for further research on the transcontinental spread of AMR.

## P-025

# Impact of the 2022 EU zinc oxide ban on antibiotics sales for group treatment of pigs in Sweden

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**Background and objectives:** Products with high-dose zinc oxide (ZnO), used in pig feed to prevent post-weaning diarrhoea in piglets, were withdrawn in the EU in June 2022 [1]. A potential consequence of the ZnO ban was an increased need to treat post-weaning diarrhoea in pigs with antibiotics, which might affect antibiotic resistance in *E. coli* from pigs. Therefore, sales of relevant antibiotic products for group treatment have been monitored closely in Sweden.

**Methods:** Monthly sales from pharmacies from 2019 to 2023 for pigs, covering products containing colistin, neomycin, or paromomycin for oral group treatment (ATCvet code QA07AA), were obtained from the Swedish eHealth Agency. Course doses were calculated per product (recommended daily dose * 12 kg * 3 days). Assuming a five-month interval between treatment and slaughter, monthly course dose sales were then divided by the number of pigs slaughtered five months later [2], and results were expressed as the average number of course doses per 1000 pigs, quarterly. Susceptibility testing of clinical *E. coli* isolates from pigs was performed as routine diagnostics, using broth microdilution.

**Results:** Sales rose in Q4 2021, increased throughout 2022, and reached a peak in Q1 2023 (Figure). Subsequently, sales steadily decreased until a marked increase broke the trend in Q4 2024. No corresponding decrease in the sales for pigs of injectable sulphonamide-trimethoprim products was observed. No clear trend in resistance to neomycin in *E. coli* could be seen.

**Conclusions:** The EU ZnO ban led to an increased need to use antibiotics for the treatment of post-weaning diarrhoea. A sales increase was observed before the ban, likely due to early attempts to discontinue the use of ZnO for prevention. The decreasing trend from Q2 2023 suggests improved implementation of farm-specific preventive measures. The interpretation of neomycin resistance

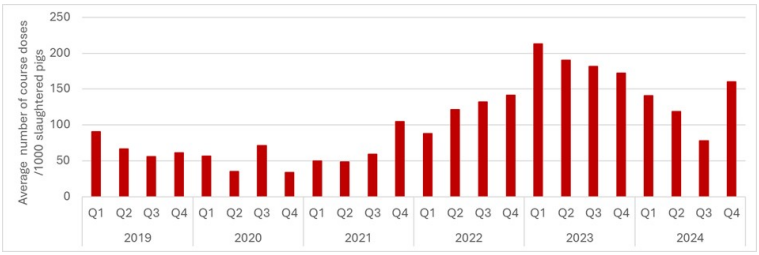
surveillance results is hampered by the low number of samples. Further monitoring of both sales and antimicrobial resistance trends is needed.

Figure. Quarterly (Q) sales of products for group medication of post-weaning diarrhoea in pigs, expressed as course doses for pigs divided by number of pigs slaughtered five months later.

References
[1] Commission Implementing Decision of 26.6.2017; C(2017) 4529 final

[2] SBA statistics: https://jordbruksverket.se/om-jordbruksverket/jordbruksverkets-officiella-statistik

**Figure**




## P-026

# Antibiotic resistance related to broiler chicken production systems in Quebec, Canada

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**Background and objectives:** Chicken farmers in Canada are restricting the use of medically important antimicrobials (MIA) to breakdown antimicrobial resistance. This study evaluated the effects of broiler chicken production systems at eight geographically dispersed farms in Quebec. Most farms were sampled three times (fall 2023, spring 2024, summer 2024).

**Methods:** Two farms from each conventional (FD and FI: CON), farms raised without MIA (FA and FB: RWMIA), raised without antibiotics (FC and FG: RWA) and organic (FE and FF: ORG) production systems were included in the study. CON farms used antibiotics of human importance, while RWMIA farms exclusively used non medically important antibiotics for humans. However, at one of the visits, farms FA, FB and FC used antibiotics to treat infections and were then classified as CON*. At each farm visit, 10 chicken caeca, 5 litter samples, 2x1L of water and 1 pool of wild bird droppings found around the chicken barns were collected. Specific bacteria were isolated from collected samples using selective media: *Enterococcus* on m-Enterococcus Agar, *E. coli* on Chromocult Coliform Agar and *Klebsiella* on Simmons Citrate Agar with 1% myo-inositol. Also, *E. coli*, *Klebsiella* and other unknown bacteria (colonies that did not appear as *E. coli* or *Klebsiella*) were isolated on selective media containing 4 µg/ml of cefotaxime (CTX). *E. coli* identification was confirmed by PCR. MALDI-TOF was also used to identify the presumptive *Klebsiella* and a few unknown CTX-resistant bacteria. The resistance of presumptive *Enterococcus* to ampicillin (AMP), tetracycline (TET) and vancomycin (VAN) was assessed by replica plating on plates containing intermediate resistance breakpoint concentrations (16, 8, 8 µg/ml, respectively). For all resistant isolates, the minimal inhibitory concentration (MIC) of 8 antibiotics was determined by a broth microdilution method.

**Results:** In total, 98 non-redundant *E. coli* resistant to CTX were isolated. The number of positive samples for *E. coli* resistant to CTX, collected in all farms between May to August 2024, is reported in the Table below. The MIC results showed that all 98 *E. coli* strains were resistant to AMP and CTX, but none to azithromycin, ciprofloxacin or meropenem. Resistance to TET was found on all farms. Gentamicin resistance was more variable, and resistance to chloramphenicol was only detected on farm FB. Among the 808 presumptive *Enterococcus* strains collected and tested with the replicator, 304 grew on TET plates. Of these, 6 were also resistant to AMP and were identified as *E. faecium* by MALDI-TOF. No *Enterococcus* was resistant to VAN. Among the 127 presumptive CTX-resistant *Klebsiella* isolates, MALDI-TOF only confirmed 8 that were *K. pneumoniae*, while most were *E. coli*. Some of the unknown CTX-resistant bacteria were identified as *Acinetobacter calcoaceticus*, *Pseudomonas sp.* and *Staphylococcus ureilyticus*.

**Conclusions:** Resistance to AMP, CTX and TET is widespread in studied broiler chicken farms in Quebec. Interestingly, the prevalence of CTX-resistant *E. coli* was high in farms that used antimicrobial treatment to control episodic infection (denoted CON* in Table). Bacterial 16S rRNA sequencing of caecum samples, will next be conducted to examine possible correlations between microbiota composition and prevalence of resistant bacteria.

**Figure**

| | FA | FB | FC | FD | FE | FF | FG | FI |
|---|---|---|---|---|---|---|---|---|
| Farm type at sampling | CON* | CON* | CON* | CON | BIO | BIO | RWA | CON |
| Caeca | 9/10 | 6/10 | 9/10 | 1/10 | 0/10 | 2/10 | 1/10 | 0/10 |
| Litter | 2/5 | 1/5 | 3/5 | 0/5 | 0/5 | 5/5 | 1/5 | 0/5 |
| Wild bird droppings | 1/1 | 1/1 | N/A | 0/1 | 0/1 | 0/1 | 0/1 | 1/1 |

**P-027**

# Novel KPC-2 Variants and Epidemic ST463 Clones Underlie Ceftazidime/Avibactam Resistance in Carbapenem-Resistant Pseudomonas aeruginosa

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**Background and objectives:** Carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) poses a significant therapeutic challenge due to limited treatment options. Ceftazidime/avibactam (CAV) is a critical antibiotic combination for multidrug-resistant infections, but emerging resistance threatens its clinical utility. This study aimed to investigate the prevalence of CAV resistance in CRPA strains, identify associated genetic mechanisms (particularly KPC variants), and evaluate the risk of CAV resistance development in KPC-2-producing CRPA under therapeutic pressure, with implications for clinical management and antibiotic stewardship.

**Methods:** A total of 273 non-duplicate CRPA isolates were collected from two tertiary hospitals in Hangzhou (2021–2022). CAV resistance was screened using agar dilution, with confirmatory susceptibility testing via micro-broth dilution. Whole-genome sequencing characterized resistance genes, virulence factors, and sequence types in CAV-resistant isolates. Conjugation experiments and plasmid stability assays evaluated the transferability and persistence of resistance determinants.

**Results:** Among 273 CRPA isolates, 13.92% (38/273) exhibited CAV resistance (MIC ≥16 μg/mL). CAV-resistant strains demonstrated extensive multidrug resistance to carbapenems, β-lactams, quinolones, and monocyclic lactams but retained partial susceptibility to aminoglycosides and polymyxins. ST463 emerged as the dominant sequence type (76.3%), exclusively associated with blaKPC-2 carriage (80.56% of carbapenemase-positive strains). These ST463(O4) clones harbored virulence factors, including type III secretion system effectors (exoS, exoU) and T6SS-associated pldA. Metallo-β-lactamase genes (blaIMP-45, blaNDM-1) were detected in 18.42% of strains. Three novel blaKPC-2 variants (KPC-14, KPC-33, KPC-86) were identified in CAV-resistant isolates, conferring high-level CAV resistance (MIC >32 μg/mL) alongside elevated carbapenem MICs (imipenem: 4 μg/mL; meropenem: 32 μg/mL).

**Conclusions:** CAV resistance in CRPA is prevalent (13.92%), driven by high-risk ST463 clones carrying blaKPC-2 variants and exhibiting multidrug resistance and enhanced virulence. The emergence of novel KPC variants under antibiotic pressure underscores the need for rigorous susceptibility monitoring and infection control measures to mitigate the spread of these pathogens. ST463 CRPA"s dual threat of resistance and pathogenicity necessitates prioritized surveillance and stewardship to preserve CAV efficacy.

**Figure**

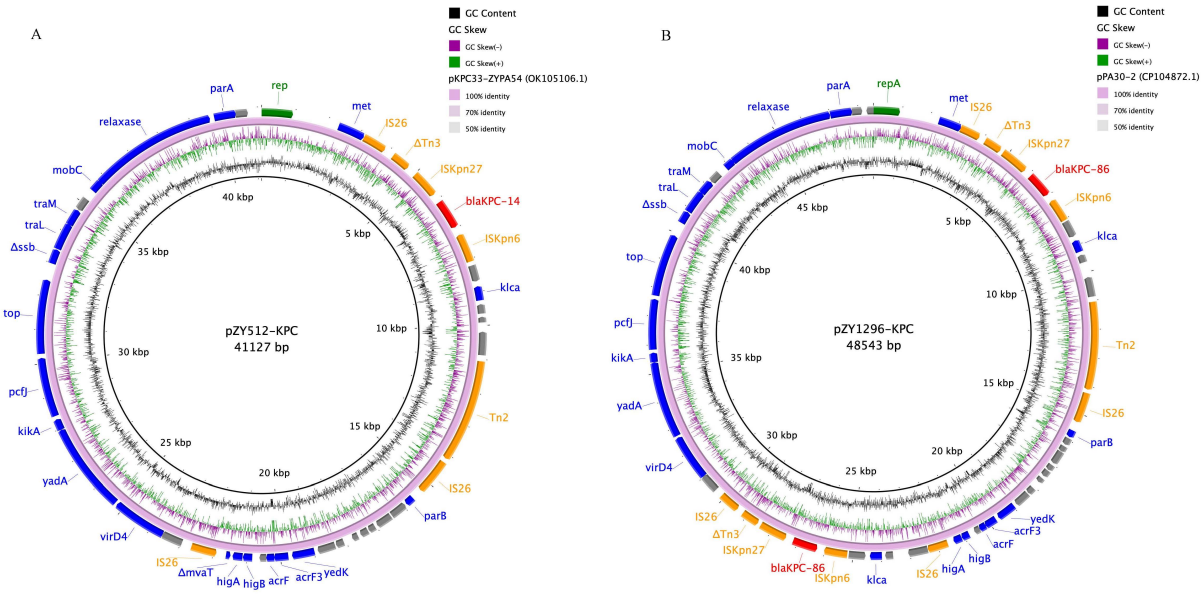


Fig. 1. Circular plasmid maps of pZY512-KPC (KPC-14)(A) and pZY1296-KPC (KPC-86) (B). Red, orange, green and blue arrows indicate the antimicrobial resistance genes, mobile elements, repA and other predicted ORFs, respectively. The names of antimicrobial resistance genes, mobile elements are labeled alongside the corresponding arrows.

## P-028

# Antimicrobial Resistance in cattle and wildlife in Namibia – a pilot study


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**Background and objectives:** Only scarce AMR data from the livestock and wildlife sector is available in Namibia. In line with the Namibian AMR National Action Plan [1], AMR monitoring activities are

jointly implemented to receive a first systematically collected data set on the AMR burden in these domains. We focused on high priority AMR bacteria according to the WHO Bacterial Priority Pathogens List [2].

References:

[1] WHO. Namibia: Antimicrobial resistance national action plan 2017. 2022 17.03.2025]; Available from: https://www.who.int/publications/m/item/namibia-antimicrobial-resistance-national-action-plan. [2] WHO, T. WHO bacterial priority pathogens list, 2024. 2024 17.03.2025]; Available from: https://www.who.int/publications/i/item/9789240093461.

**Methods:** A protocol for sample collection, labelling and lab analysis was established. The sampling sites were all located in the Windhoek district within the Khomas region. The sites included one cattle farm, one cattle feedlot and one wildlife farm. Individual fecal samples were collected, while water samples were collected exclusively from the wildlife farm. Samples were investigated for ESBL-*E. coli,* methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant Enterococci (VRE) applying a culture-based approach using selective chromogenic media. Antimicrobial susceptibility testing (AST) via Vitek 2 was performed according to CLSI standards.

**Results:** In total, 167 fecal and 5 water samples were collected between June and September 2022. 137 samples were taken on the farm/ feedlot, and 35 on the wildlife site (incl. 5 water samples from water holes). In general, 76.5% of Gram-positive bacteria (42%) belonged to *Enterococcus* spp. and 23.5% to *Staphylococcus* spp. 4% of the samples revealed Gram-negative bacteria (mostly *Sphingomonas paucimobilis*). In cattle samples, 59.7% were *Enterococcus* spp. and 28.4% were *Staphylococcus* spp., of which 72% were *S. aureus.* 28.3% of *Enterococcus* spp. showed acquired resistance mainly against fluoroquinolone antibiotics. 5.1% of the Enterococci were VRE. All *S. aureus* isolates were MRSA positive and multidrug-resistant (MDR) with 61.5% resistance to trimethoprim/sulfamethoxazole followed by clindamycin (38.5%). In wildlife samples 3.2% were *Staphylococcus* spp. and 80.6% were *Enterococcus* spp. 18.3% of the latter showed acquired resistance (100%) to fluoroquinolones and 36% to glycopeptide antibiotics. 9.5% of the Enterococci were VRE. No AMR bacteria of interest were found in water samples. Analysis of the Gram-negative samples is ongoing.

**Conclusions:** The results of this small-scale pilot study show that AMR bacteria are present in Namibia's animal and wildlife sector. AMR bacteria listed as high priority in the WHO Bacterial Priority Pathogens List, such as MRSA [2], could be identified in the farm setting. Resistance to over-the-counter antibiotics, such as trimethoprim/sulfamethoxazole, is most common in MDR *S. aureus* isolates. Interestingly, VRE was more frequently found in wildlife than on farms. Holistic approaches involving comprehensive data acquisition, awareness raising, underpinned by meaningful policies, are needed to combat AMR efficiently.

## P-029

# Identification of bacteria at the umbilicus of newborn calves and in case of umbilical infections and their susceptibility to antimicrobial agents commonly used

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**Background and objectives:** Umbilical infections (UIs) are one of the most common diseases in neonatal calves, initiated around birth by environmental, fecal, skin und mucosal bacteria. Bacteria colonizing the umbilical cord after birth and those involved in UIs were tested for their susceptibility to antimicrobial agents widely used.

**Methods:** Five dairy farms in Brandenburg (Germany) were visited for twelve consecutive weeks, between September 2022 and April 2024. Information regarding the substance used for navel care treatment and the treatment strategy of UIs were gathered. All calves between 1 and 28 days were clinically examined on a weekly basis including the examination of the umbilicus for signs of inflammation (enlargement, heat, tough consistency, pain, discharge). Calves suffering from an UI that were accessible for sampling (e.g., discharge, fistula) or calves born during the farm visits were sampled using swabs. After bacterial cultivation, species identification was carried out using MALDI-TOF MS. Antimicrobial susceptibility testing by broth microdilution was performed according to CLSI standards for *Escherichia coli, Trueperella pyogenes, Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae,* and *Klebsiella* spp..

**Results:** In 106 samples from calves with UIs and 47 samples from newborn calves, more than 100 different bacteria were identified with significant differences between both groups. *T. pyogenes, S. aureus, S. uberis* and *S. dysgalactiae*, as well as anaerobic bacteria, were almost exclusively isolated from UI samples. More than two-thirds of all *S. aureus* isolates investigated were methicillin-resistant. *Streptococcus* spp. and *T. pyogenes* showed high susceptibility to antibiotics commonly administered in UI cases (penicillin/benzylpenicillin). Moreover, all pathogens tested were susceptible to amoxicillin/clavulanic acid. Low susceptibilities to tetracycline, however, were found for all pathogens tested, except for *Klebsiella* spp.. Calves with UIs from farms using chlortetracycline-spray for navel care showed significantly higher MICs for most antimicrobial agents tested compared to farms using iodine. *E. coli* isolated from calves with UIs had significantly higher MICs for nearly all tested antimicrobial agents compared to *E. coli* from newborn calves without UIs.

**Conclusions:** Besides the diversity of bacterial species isolated from UIs, a change of the bacterial spectrum and a decrease in their susceptibility in UI cases compared to pathogens from newborn calves could be demonstrated. Furthermore, the risk of a possible promotion of antimicrobial resistance induced using chlortetracycline-spray for navel care could be shown.

## P-030

# Antimicrobial Resistance in Horses in Finland

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**Background and objectives:** Antimicrobial resistance (AMR) in horses is an emerging concern in veterinary medicine, posing challenges for effective treatment and potential risks to both humans and the environment.[1] Resistant bacterial strains can be transmitted to humans, particularly those working with horses, and may contribute to environmental contamination through the excretion of resistant bacteria into soil and water.[1] [2] Despite the global recognition of AMR as a critical issue, comprehensive studies on the current state of AMR in horses in Finland are lacking. Understanding trends in the resistance profiles of equine pathogens is essential for establishing treatment protocols and guidelines for the prudent use of antimicrobials. The objective of this study was to evaluate the development of AMR in equine pathogens over an 11-year period.

**Methods:** This retrospective study analyzed antimicrobial susceptibility data from bacterial isolates obtained from clinical equine samples submitted to the Clinical Microbiology Laboratory of the Veterinary Teaching Hospital, University of Helsinki, between 2013 and 2023. The study focused on the most significant or frequently occurring equine pathogenic bacteria: *Streptococcus* sp., *Staphylococcus* sp., *Enterobacterales* (excluding *Escherichia coli*), *Escherichia coli*, and *Actinobacillus* sp. Antimicrobial susceptibility testing was performed using standardized methods, and only antimicrobials commonly used in equine medicine in Finland or relevant to the bacterial group were included in the analysis. The development of resistance over time was assessed using trend analysis to identify significant changes in antimicrobial susceptibility patterns across the study period.

**Results:** Chi-square analysis for trend showed that *Streptococcus* sp. exhibited a significant increase in resistance to trimethoprim/sulfamethoxazole (TMS) ($p$ = 0.002, slope = 0.0083) over the evaluated period. *Enterobacterales* demonstrated a significant decrease in resistance to doxycycline ($p$ < 0.0001, slope = -0.0715), enrofloxacin ($p$ = 0.0001, slope = -0.0379), gentamicin ($p$ < 0.0001, slope = -0.055), and TMS ($p$ < 0.0001, slope = -0.0582). *Actinobacillus* sp. showed a significant increase in resistance to tetracycline ($p$ = 0.0408, slope = 0.035).

**Conclusions:** This study highlights significant trends in antimicrobial resistance among bacterial isolates from equine clinical cases in Finland over an 11-year period. The findings indicate both increasing and decreasing resistance patterns, emphasizing the dynamic nature of AMR. The increased resistance of *Streptococcus* sp. to TMS and *Actinobacillus* sp. to tetracycline suggests a need for prudent antimicrobial use and continuous monitoring. However, the decreased resistance in *Enterobacterales* to multiple antimicrobials is an encouraging trend. These results underline the importance of ongoing AMR surveillance and responsible antibiotic prescribing in equine medicine to mitigate the impact of AMR and safeguard both animals and humans.

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## P-031

# Prevalence and diversity increases of Extended-Spectrum Cephalosporin-Resistant *E. coli* in Dutch livestock

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**Background and objectives:** Previously, the monitoring of Extended-Spectrum Cephalosporin-Resistant (ESC-R) *Escherichia coli* in the Netherlands, such as ESBL-producing *E. coli*, has predicted only a minor part of ESC-R *E. coli* in humans to be attributed to livestock. [1] Over the past decade, the type of ESC-resistant *E. coli* detected in livestock species fluctuated. [2] Resistant genes are detected at varying levels over time, indicating that the attribution from livestock to humans, and vice-versa, might have changed over time.

**Methods:** Samples from the Dutch monitoring program for antimicrobial resistance in livestock were selectively cultured for ESC-R *E. coli*. ESBL and AmpC resistance mechanisms were detected by PCR or whole genome sequencing and compared from 2014 to 2024.

**Results:** In the past decade, the prevalence of ESC-R *E. coli* has decreased in broilers, remained stable in pigs and has increased in veal calves and dairy cattle. Fluctuations in the prevalence of resistance genes over time was detected in most livestock species. In broilers, *bla*CMY-2 has been replaced by *bla*CTX-M-1 and *bla*SHV-12. In dairy cattle, *bla*CTX-M-15 was first detected in 2015 and is now most predominantly present in 40% of ESC-R *E. coli*. *bla*CTX-M-15 is also detected in veal calves, but an increase in *bla*CTX-M-32 has been detected since 2021. Only in pigs, relatively stable proportions were detected of mutations in the promotor chromosomal ampC and *bla*CTX-M-1.

Analysis of the WGS data indicates related *E. coli* are detected in each of the livestock sectors. However, the increasing detection of *bla*CTX-M-32 is caused by clonal expansion of a ST1433 lineage, primarily detected in white veal calves, but also in smaller numbers in rosé veal calves, one dairy cow and one pig isolate.

**Conclusions:** Source attribution studies between livestock and humans in the Netherlands should be updated with recent data. Specifically, WGS data is much more readily available and will give further insight. Additional studies are needed to determine the cause of increasing prevalence of ESC-R *E. coli* and clonal expansion in veal calves and dairy cattle.

**References**

[1] L. Mughini-Gras *et al.* Attributable sources of community-acquired carriage of Escherichia coli containing β-lactam antibiotic resistance genes: a population-based modelling study, 2019, DOI: 10.1016/S2542-5196(19)30130-5

[2] MARAN 2024, Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2023, www.wur.nl/maran

## P-032

# Novel SCC*mec* element containing two *mecA* copies identified in human MRSA ST398

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**Background and objectives:** MRSA ST398 is mainly associated with livestock especially pigs, cattle and horses, and also many colonized humans working with animals. However cases of infections may occur in both animals and humans. MRSA harbor a *mec* gene on Staphyloccocal cassette chromosome *mec* (SCC*mec*) encoding a penicillin-binding protein (PBP2a) with low affinity for beta-lactams antibiotics1. The aim of this study was to characterize a novel SCC*mec* element using WGS-based analysis of a MRSA isolated from nasal cavity of a human in Spain.

**Methods:** Complete genome sequence was obtained from long fragment Illumina libraries sequenced on PacBio platform. The long reads were assembled using Autocycler v0.2.1 and annotated with Bakta v1.11.0. Core genome multilocus sequence typing (cgMLST) and *spa* typing were performed using Ridom SeqSphere+ v10.0.4. Antimicrobial resistance were screened using ABRicate version 1.0.1 against ResFinder version 4.6 database. SCC*mec* was analyzed manually.

**Results:** MRSA strain C1989 belonged to ST398 *spa* type t1197. It harbored a novel SCC*mec* element wich contains two *mecA* genes which were 100% similar to each other. This SCCmec element consisted of a combination of SCC*mec* types V from *S. aureus* strain TUM9463 (AP019306.1) and SCC*mec* VIIKM241 *S. pseudintermedius* strain KM241 (AM904731). Strain C1989 carried additional antimicrobial resistance genes including *blaZ, ant*(9)-*Ia, aadD1, vga*(A)*, lmrS, tet*(L), *tet*(M), and *dfrK.*

**Conclusions:** The presence of a novel hybrid SCC*mec* element carrying two identical *mecA* genes MRSA indicated that new sublineages of ST398 may occur and are present in humans.

**P-033**

# Antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* from small ruminants in Portugal: detection of *mecA* and *mecC* in sheep

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**Background and objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known public health concern due to its resistance to multiple antibiotics and potential for zoonotic transmission. The prevalence and antimicrobial resistance of MRSA from small ruminants remain scarce. Here, we characterized the MRSA population isolated from sheep and goats in the scope of a pilot surveillance antimicrobial resistance project.

**Methods:** Nasal swabs were collected from animals from 72 sheep farms and 34 goat farms between 2024 and 2025 in pools of six animals per farm. Presumptive MRSA were isolated on *Brilliance*™ MRSA 2 Agar and confirmation was performed by antimicrobial susceptibility testing using the microdilution method. Results were interpreted according to EUCAST ECOFFs. Multiplex PCR was performed for the detection of *mecA*, *mecC*, *spa*, and PVL genes.

**Results:** Presumptive MRSA were detected in ten pools (9.4%, 10/106): 6.9% (5/72) from sheep and 14.7% (5/34) from goats. All presumptive MRSA isolates were susceptible to erythromycin, fusidic acid, gentamicin, kanamycin, linezolid, mupirocin, streptomycin, rifampicin, sulfamethoxazole, quinupristin/dalfopristin, trimethoprim, and vancomycin. In sheep, two isolates were resistant to cefoxitin, MICs of 8 and 16 µg/mL, harbouring *mecC* and *mecA*, respectively. These two isolates were multidrug-resistant with resistance to chloramphenicol and tetracycline; the *mecA*-positive isolate was also resistant to tiamulin, clindamycin, ciprofloxacin and penicillin. Three isolates from goats were resistant to cefoxitin (MIC=8 µg/mL), without *mec* genes detection, indicating a possible borderline resistance.

**Conclusions:** To our knowledge, this is the first report of *mecC* MRSA in sheep from Portugal. This study shows the importance of surveillance in small ruminants for understanding the current epidemiological situation and promoting responsible antimicrobial use. Further studies on cefoxitin-borderline resistance are needed, given the implications for the diagnosis and treatment.

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**P-034**

# Emergence of Carbapenemase-producing *Escherichia coli* isolated from swine in Portugal

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**Background and objectives:** Carbapenems are a group of beta-lactams considered reliable for treating serious human infections caused by multidrug-resistant (MDR) bacteria. Carbapenemase-producing Enterobacteriales (CPE) are a worrisome public health risk due to their MDR nature and potential for disseminating genes by horizontal gene transfer. Here, we report the characterization of carbapenem-resistant *Escherichia coli* isolates recovered in 2023 from food-producing animals under the scope of the national surveillance program on monitoring antimicrobial resistance (EU 2020/1729).

**Methods:** A total of 334 swine cecal samples were plated onto BBL™ CHROMagar™ CPE culture medium to search for carbapenemase *E. coli* producers. Antimicrobial susceptibility of the isolates was confirmed using broth microdilution (EUVSEC3 and EUVSEC2 microplates, Sensititre), and the results were interpreted according to ECOFF values established by EUCAST. Genetic characterization of isolates was performed through WGS, and the genetic context was assessed using freely available Bioinformatics tools.

**Results:** Seven carbapenem-resistant *E. coli* isolates were obtained. All showed high levels of resistance to temocillin (MIC >128 mg/L) and carbapenems: ertapenem (MIC>2 mg/L), meropenem (MIC from 1mg/L to >16 mg/L) and imipenem (MIC from 2mg/L to 16 mg/L). Five isolates from ST410 harbored *bla*OXA-181, and four (n=4) also carried *bla*NDM-5,*bla*OXA-1 and *bla*CMY-2 genes. Two isolates belonging to ST2380 and ST224 held *bla*OXA-244/*bla*CTX-M-65 and *bla*OXA-48/*bla*CMY-2 genes, respectively. The main plasmids found were ColKP3, IncX3, IncFII (pAMA1167-NDM-5) and Col (BS512).

**Conclusions:** To our knowledge, this is the first time that carbapenemase-producing *E. coli* has been isolated from swine in Portugal. The emergence of MDR high-risk carbapenemase-producing *E. coli* ST410 clones from human and animal sources is being reported worldwide, becoming recognized as a global health threat [1,2]. Food-producing animals seem to play an important role as reservoirs of carbapenem-antimicrobial resistance determinants showing the importance of permanent surveillance and control programs.

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**P-035**

# The emergence of *tet*(X4)-positive *Klebsiella* spp. in aquatic products

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**Background and objectives:** Tigecycline, a crucial last-resort antimicrobial, faces potential ineffectiveness due to the emergence of the *tet*(X4) gene [1]. The *tet*(X4) gene is predominantly found in *E. coli* and has been sporadically detected in porcine and clinical *K. pneumoniae* isolates [2]. Notably, *tet*(X4) has not yet been identified in *Klebsiella* spp. strains from aquatic sources. This study aimed to explore the prevalence and characteristics of *tet*(X4)-positive *Klebsiella* spp. strains isolated from aquatic products in Hainan Island of China.

**Methods:** A total of 442 aquatic product samples were collected from 126 wet markets and 5 supermarkets across Hainan Island, between 2023 and 2024. Samples were homogenized in BPW, pre-enriched in LB broth, streaked onto CHROMagar™ Orientation medium, and selected colonies were further incubated. The presence of the *tet*(X4) was screened by PCR and Sanger sequencing. Antimicrobial susceptibility testing was performed according to CLSI and FDA (for tigecycline) guidelines [3, 4]. Whole-genome sequencing was carried out using Illumina NovaSeq 6000 platform.

**Results:** Fourteen *tet*(X4)-positive *Klebsiella* strains were identified, including 10 *K. pneumoniae* and 4 *K. quasipneumoniae*. The detection rate in fish (4.0%) was higher than shellfish (2.8%) and shrimps (2.4%). Thirteen of these 14 isolates were multidrug-resistant, exhibiting resistance to up to seven classes of antimicrobials. All isolates were resistant to florfenicol, doxycycline, and tigecycline, while remaining susceptible to colistin, meropenem, ceftazidime-avibactam, and amikacin. In addition, most isolates were resistant to commonly used aquatic antimicrobial agents, including ampicillin (92.9%), trimethoprim-sulfamethoxazole (92.9%), and enrofloxacin (71.4%). The maximum likelihood tree constructed from these 14 *tet*(X4)-positive isolates showed diverse genetic backgrounds, belonging to different ST types and K locus types, with only KL147 appearing in multiple isolates. Conjugation experiments showed that 10 of these isolates were able to transfer antimicrobial resistance genes to the recipient *E. coli* C600 at frequencies ranging from $9.3\times10^{-5}$ to $3.2\times10^{-3}$.

**Conclusions:** This study reports the first detection of *tet*(X4)-positive *Klebsiella* spp. in aquatic products, indicating the potential role of seafood as a reservoir and transmission vector for tigecycline resistance. The isolates exhibited extensive multidrug resistance and genetic diversity, and many were capable of transferring resistance via conjugation. These findings underscore the urgent need for enhanced monitoring and control measures in the aquaculture industry to mitigate public health risks.

**P-036**

# Analysis of antimicrobial resistance genes present in *Escherichia coli* isolated from irrigation water sources

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**Background and objectives:** Irrigation water contaminated with bacterial pathogens carrying antibiotic-resistant genes (ARG), can lead to contamination of fresh vegetable produce [1]. This poses significant public health risks, particularly if the produce is consumed in its raw form. *Escherichia coli* is a common environmental bacterium and food pathogen known for harbouring multidrug resistance genes. In this study, the presence of ARGs was investigated in *E. coli* isolates collected from water sources used for irrigation of vegetable crops.

**Methods:** Irrigation water samples (N=250) were collected over twelve months from seven water sources, including five rivers and two canals. The samples were screened for the presence of *E. coli* and a subset of sixty-two isolates were selected for further characterisation. Whole genome sequencing was performed using Oxford Nanopore MinION long-read sequencing and bioinformatic analysis was undertaken to assemble the bacterial genomes and determine the presence of ARGs and heavy metal resistance genes.

**Results:** All sixty-two isolates had intrinsic resistance genes to beta-lactams (*ampC* and *ampH*) along with zinc and copper resistance genes. Additionally, nine *E. coli* isolates also harboured tetracycline, sulfonamides, aminoglycosides, trimethoprim, florfenicol, fluoroquinolones, macrolides and other beta-lactams resistance genes. The tetracycline resistance gene was observed in eight out of the nine isolates and seven had ARGs against two or more antibiotics. Heavy metal resistance genes (MRGs) against arsenic were detected in fifty-seven isolates, in addition to silver resistance gene in three isolates and mercury resistance in two isolates.

**Conclusions:** The detection of multi-drug resistant *E. coli* isolates in horticulture irrigation water indicates the presence of ARGs within water resources and highlights the need for further research to determine their role in ARGs transmission. Moreover, the presence of ARGs and MRGs suggests possible co-selection between antimicrobial and heavy metal resistance. These findings provide a baseline for future studies and emphasize the importance of monitoring environmental samples such as water resources to track emerging AMR trends.

**Future study**: Antimicrobial susceptibility testing will be performed to confirm the phenotype of the *E. coli* isolates and evaluate the risk associated with their sources.

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**P-037**

## Major animal species, minor bacterial pathogens: a bacterial minority report from different clinical cases in pigs, 2020-2024

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**Background and objectives:** GD performs antimicrobial susceptibility (AMS) testing of pathogens from different animal species, both to guide therapeutic treatment and for monitoring purposes. Since the implementation of MALDI-TOF MS for identification of bacteria, the range of bacterial species identified at the laboratory of GD has broadened. The objective of the present study was to review the different "minor" bacterial species identified and their AMS.

**Methods:** The required information (bacterial species (minimum number of isolates per species: 15) and AMS results) was extracted from the Laboratory Information System, with all isolates originating from clinically diseased pigs submitted to the necropsy room of GD from 2020-2024. Regarding AMS, the Gram-negative test panel covered 19 different active compounds and the Gram-positive panel 11. At post-mortem examinations, macroscopic lesions were sampled, and hence only bacterial species apparently associated with these macroscopic lesions were isolated and identified.

**Results:** The most frequently observed "minor species" included, *Klebsiella pneumoniae* (n=22), *Staphylococcus chromogenes* (n=16), *Streptococcus dysgalactiae* ssp. *dysgalactiael* (n=17), and *Streptococcus dysgalactiae* ssp. *equisimilis* (n=145). These bacteria were cultured from various organs and identified by the pathologist as causative agent.

To provide prudent, practical treatment advice, GD primarily focuses on first choice antimicrobials (according to the Dutch classification). Generally, isolates were characterized by low MICs of first choice antimicrobials tested. However, for both *Streptococcus* subspecies high MIC values were obtained for clindamycin (indicator of lincomycin) (±30-40% of the isolates), erythromycin (indicator of tylosin) (±30-40%), and tetracycline (±60-70%). *S. chromogenes* (±40%) also had high tetracycline MICs. *S. chromogenes* (±25%) additionally had high MICs of penicillin. *K. pneumoniae* isolates revealed relatively high MICs of florfenicol (all), spectinomycin (±40%), streptomycin (±30%), and tetracycline (±30%).

**Conclusions:** Several of the identified species are known from literature to be clinically relevant in pigs. However, as interpretation of MICs for these minor pathogens is hampered by the lack of CLSI/VetCAST-defined clinical veterinary breakpoints, it is very difficult to provide practitioners with prudent practical treatment advice. Pig-specific clinical breakpoints are available for only five bacterial species, for only respiratory disease. More veterinary breakpoints are needed for responsible and prudent use of antimicrobials.

**P-038**

## Utilising *E. coli* as an indicator for the surveillance of antimicrobial resistance in Integrated Constructed Wetlands

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**Background and objectives:** Antimicrobial resistance (AMR) is acknowledged as one of the greatest challenges to human health globally. Conventional wastewater treatment is often considered a hotspot for AMR and the release of antimicrobial resistant organisms (AROs) into the natural environment. This study focused on the efficacy of Integrated Constructed Wetlands (ICWs) to remove AROs in a range of sectors. *E. coli* is considered a useful indicator for AMR surveillance in the environment given the availability of established methods and their cost effectiveness. The objective of this study was to assess the antimicrobial resistance profile of *E. coli* from ICWs and to determine the impact of the isolation media used on the AMR profiles of the isolates obtained.

**Methods:** The antimicrobial susceptibility profile for 16 different antimicrobials was determined by disk diffusion for a bank of 380 *E. coli* isolates obtained from the influent or effluent of seven different ICWs in Ireland. The profiles were compared for *E. coli* isolates obtained from various antimicrobial containing selective media (n=240) and *E. coli* isolates obtained from selective media without antimicrobials (n=140).

**Results:** A variety of different antimicrobial susceptibility profiles were observed in the bank of isolates, with multi drug resistance observed in a number of isolates. The isolates obtained from antimicrobial containing selective media encoded higher levels of resistance than the isolates obtained from the media which did not contain antimicrobials.

**Conclusions:** This study demonstrated the value of utilizing *E. coli* as an indicator for specific resistance phenotypes, but also more broadly for AMR in the environment. The choice of isolation media influences the AMR profile of the isolates obtained and this needs to be considered when designing surveillance studies, interpreting the results and comparing with other studies.

**P-039**

## Detection of Extended spectrum beta-lactamase (ESBL) from Raw Beef and Chicken Supplied to Fast Food Restaurants and Hotels in Abuja, Nigeria

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**Background and objectives: Background and Objectives:** The true burden of antimicrobial resistance (AMR) in food animals and its threat to public health in low to middle-income countries (LMICs) is poorly defined. Furthermore, there is limited information on the burden of AMR including to medically important drugs amongst animals and their products in Nigeria. A cross-sectional study was carried out to determine the prevalence of contamination of raw meat with extended-spectrum b-lactamase producing (ESBL) *Escherichia coli* for raw meat used for food served in restaurants in Abuja, Nigeria.

**Methods: Methods:** A total of 280 samples were screened for ESBL-producing *E. coli* represented by 137 raw beef samples and 143 raw chicken meat samples, which were collected from 151 fast food restaurants and hotels in Abuja. Sampling was carried out by a systematic random sampling approach. The sampling frame was based on the list of restaurants provided by the Association of Fast Food and Confectioneries in Nigeria (AFFCON) Abuja Chapter. Samples were cultured using isolation media, eosin methylene blue agar (EMBA) with overnight incubation for pre-enrichment (Peptone water) and then incubated overnight in modified Tryptone soy broth before plating on EMBA. One to two presumptive *E. coli* colonies were picked and identified using conventional biochemical

methods and Microbact 12E identification kit. The *in-vitro* susceptibilities to 20 commonly used antimicrobial agents(tetracycline 30µg(TET), amoxicillin/clavulanic acid 30µg(AMC), ampicillin 10µg(AMP), chloramphenicol 30µg(C), tri methoprim 5µg(W), sulphamethoxazole/trimethoprim 25µg(SXT), gentamicin 10µg(CN), ciprofloxacin 5µg(CIP), nitrofurantoin 300µg(F), amoxycillin (10µg), cephalothin 30 µg(KF), cefoxitin 30µg(FOX), cefotaxime30µg(CTX), ceftazidime 30µg(CAZ), tobramycin10µg(TOB), amikacin 30µg(AK), norfloxacin 10µg(NOR), nalidixic acid 30µg(NAL), streptomycin 10µg(STR) and Imipenem 10µg(IMP) by disc diffusion using human clinical breakpoints according to the method of Clinical Laboratory Standards Institute, (CLSI) USA was determined for *E. coli* isolates. ESBL screening was carried out using the Double disk synergy test.

**Results: Results:** In this study, Multiple drug resistance (MDR) was detected in 66.7% of isolates recovered (8/12 isolates) with one isolate exhibiting resistance to 17 antimicrobial agents and seven (7) isolates resistant to 1 or more antibiotics in more than 3 classes of antibiotics. Isolates were predominantly resistant to routine antibiotics (AMP=41.7%, TET=50%, STR=33.3%, STX=41.7%) but also exhibited resistance to aminoglycosides (TOB=25%) quinolone (NAL=25%) and cephalosporins (KF=100%, CTX=41.7%, CTZ=25%).It is worthy to note that 6 out of these isolates were from beef while 2 were from chicken sources.ESBL genes were detected genotypically at a rate of 58.3%, the ESBL beta-lactamase genes detected were *bla*TEM (33.3%), *bla*SHV (33.3%) and *bla*CTX-M (16.7%). ESBL was detected phenotypically at a rate of 66.7% with 7 isolates exhibiting MDR and ESBL concurrently both phenotypically and genetically.

**Conclusions: Conclusions:** This study highlights the presence of ESBLs and MDR in animal products meant for human consumption. This poses a significant risk in the spread of antibiotic-resistant bacteria and antibiotic-resistant genes at multiple stages in the food production chain. Competent authorities need to institute measures to reduce the prevalence and regulate the use of antibiotics in agriculture and to promote the use of food safety management systems (FSMS) to promote hygiene in food establishments.

**Figure**

Table 1: Antibiotic resistance patterns and multiple antibiotic resistance index of individual *Escherichia coli* isolated from different sample types

| ***E. coli* ID No.** | **Sample type** | **Antibiotic resistance pattern** | **ESBL phenotype** | **ESBL genes** |
|---|---|---|---|---|
| G15B | Beef | CAZ, KF | negative | |
| G33C | Chicken | CTX, KF, NAL, STR, SXT, TET, W | negative | |
| G35B | Beef | AMP, KF, TOB | positive | $bla_{CTX-M}$ |
| G44B | Beef | AK, CAZ, CTX, KF, F, STR, TET | positive | $bla_{TEM}$ |
| G46B | Beef | AMP, AML, CTX, KF, STR, SXT, TET, W, | positive | $bla_{TEM}$ |
| W1B | Beef | KF | negative | |
| W7C | Chicken | AMC, AMP, AML, CAZ, C, CIP, CTX, CN, F, FOX, KF, NAL, NOR, SXT, TET, TOB, W | positive | |
| W70B | Beef | AMP, C, F, KF | positive | $bla_{TEM}$, $bla_{SHV}$ |
| W72B | Beef | KF | positive | $bla_{SHV}$ |
| GW6C | Chicken | C, KF, TET | positive | $bla_{TEM}$, $bla_{SHV}$ |
| GW9C | Chicken | KF, SXT | negative | |
| GW13B | Beef | AMC, AMP, CTX, KF, NAL, STR, SXT, TET, TOB, W | positive | $bla_{CTX-M}$ |

**P-040**

## Digitalisation of the Norwegian monitoring programme for antimicrobial resistance in the veterinary sector

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**Background and objectives:** The Norwegian monitoring programme for antimicrobial resistance in the veterinary sector (NORM-VET) was established in 2000. Data from the programme have been recorded for more than two decades, initially using WHONET, but after some years using the internal electronic journal system (PJS) at the Norwegian Veterinary Institute. Over the years, there have been several changes in what has been included in NORM-VET, with regards to animal species, sample material, bacterial organisms, susceptibility testing panels, and substances included in these. In addition, the epidemiological cut-off values (ECOFFs) that have been applied have changed over time. Our objectives were to: 1) simplify and transfer the relevant data into a new database, and 2) establish a digital application for visualization, called NORM-VET Explorer (currently only in Norwegian).

**Methods:** We developed an R package, called "noRmvet", with functions to handle the NORM-VET data. A shiny application, the "NORM-VET Utforsker, has been developed to make the data digitally available.

**Results:** The original data have been classified to groups of animal species, material categories, bacterial categories (e.g. indicator, zoonotic, clinical, important) and bacterial groups (species). Further, we decided to include a new variable called "report_year" to align the data with the NORM/NORM-VET reports. Currently, the application only includes the indicator bacteria *Escherichia coli* and *Staphylococcus felis* and *S. pseudintermedius* from healthy carriers. However, work is still ongoing to include more data from the monitoring programme. The database is still missing data from the years where WHONET was used (2000-2003) as it has been difficult to harmonize with the data from the PJS.

**Conclusions:** Developing the NORM-VET database and its digital application NORM-VET Explorer is an ongoing process. New data from the PJS needs to be transferred yearly. In addition, variable names and ECOFFs in the new database also needs to be updated annually. Overall, however, the harmonized new database will save time and effort in the writing of future NORM-VET reports. Moreover, the NORM-VET Explorer application will have an added value for several end users (governmental stakeholders, industry and other researchers) as it allows for interactive exploration and downloading of figures, aggregated data and results. On average, the number of visitors to the NORM-VET Explorer is currently at 56 the last 30 days (as per 24.03.25). We expect this number to increase as we release further content in the application.

**References**: NORM-VET Utforsker

**P-041**

## Characterization and distribution of IME_Rho_tet, a mobile genetic element involved in the dissemination of *tet*(W) and *tet*(32) in animal and human gut microbiota

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**Background and objectives:** Ribosomal protection proteins (RPPs) conferring resistance to tetracyclines, and especially *tet*(W) and *tet*(32), are among the most abundant resistance genes in livestock animal and human gut microbiota [1,2]. These two genes were detected in a number of different bacterial species [3,4], suggesting a spread through mobile genetic elements (MGE). Nonetheless, the extent of their dissemination in gut bacteria and which MGEs are involved are still unknown. In a previous study [5], a putative *tet*(W)-carrying MGE family, hereafter called IME_Rho_tet, was identified in bacteria from human and pig gut microbiota, suggesting that it may be a key player in the dissemination of *tet*(W). In the present study, the diversity of IME_Rho_tet elements and their involvement in the spread of *tet*(W) and other RPPs in gut-associated bacteria was investigated.

**Methods:** *tet*(W) and IME_Rho_tet marker genes were screened in all *Bacillota* and *Actinomycetota* assembled genomes of the RefSeq database, using blastP with a relaxed similarity threshold of 50%. Genomes with positive hits were analyzed in more details to characterize the genetic diversity of IME_Rho_tet elements, their boundaries and preferred integration site, and their distribution among gut-associated bacteria compared to the whole RPPs distribution.

**Results:** A complete IME_Rho_tet element was detected in 496 of the 64,366 screened genomes. They ranged between 9856 bp and 17903 bp long and show a very high nucleotide diversity in their structurally conserved backbone, suggesting that they could be considered as a new MGE family. Surprisingly, 185 IME_Rho_tet elements (37%) carried the *tet*(32) gene or a novel putative RPP in place of *tet*(W). In total, IME_Rho_tet were found in 115 different bacterial genera, most being commensal of the gut microbiota. By contrast, the 1262 detected *tet*(W), 69 *tet*(32), and 3 other RPPs not associated with complete IME_Rho_tet were distributed among 136 genera of gut and non gut bacteria, with 40% also harboring IME_Rho_tet. The 5' boundary of IME_Rho_tet was observed directly upstream of many of the lonely *tet*(W), suggesting that they likely originated from this MGE.

**Conclusions:** Our results show that the IME_Rho_tet family contributed significantly to the spread of *tet*(W) and *tet*(32) among gut-associated bacteria. Future analysis of IME_Rho_tet elements in various metagenomic datasets will inform us on their link with *tet*(W) and *tet*(32) abundances in gut microbiota.

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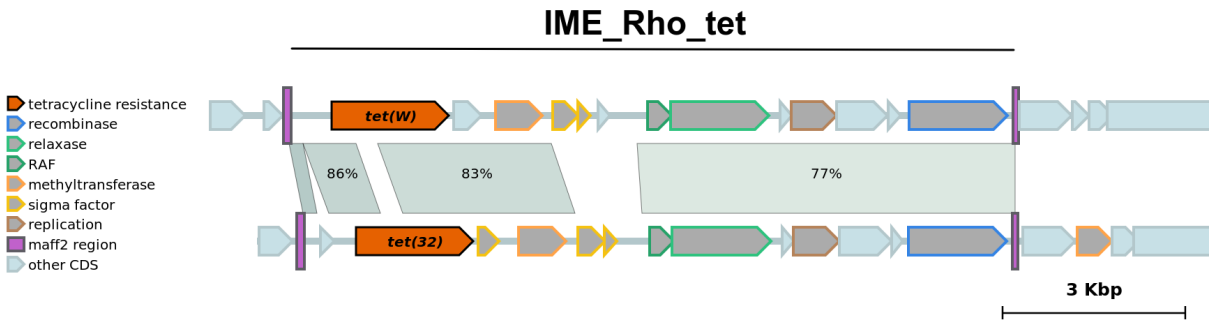
**Figure**



**P-042**

# Occurrence of Enterobacterales producers of carbapenemases in clams from the Central Adriatic Sea

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**Background and objectives:** Antimicrobial resistance (AMR) is a significant global health threats and a complex phenomenon, that requires a multidisciplinary approach. Carbapenem-resistant Enterobacterales are in the critical group of WHO Bacterial Priority Pathogens List (1). AMR monitoring programs in the EU focus on terrestrial animals. Human- and animal- gut bacteria can enter aquatic environments via various routes. Bivalves are filter-feeder animals capable to accumulate contaminants, including bacteria, which can also be resistant to antimicrobials. The aim of this study was to investigate the occurrence of carbapenemase-producing Enterobacterales (CPE) in clams (n=123) collected from harvesting areas of the Central Adriatic Sea.

**Methods:** Overall, between January 2024 and February 2025, 123 samples of clams were collected from 22 sampling points located in harvesting areas of the central Adriatic Sea. After an enrichment in buffered peptone water at 37 °C per 24h, suspensions were streaked over the surface of MacConkey Agar plates supplemented with ertapenem and chromogenic selective agar plates, which were incubated at 37° C ± 1° C for 24 h ±2 h. Isolated colonies were identified by using a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and evaluated for carbapenemase production by the modified Hodge test. Detection of *bla*KPC, *bla*VIM, *bla*NDM and *bla*OXA-48 genes was performed by a multiplex PCR using primers and conditions previously described (2).

**Results:** Overall, CPE were isolated from 6 (4.8%) of 123 samples of clams. In particular, carbapenemase-producing *Escherichia coli*, *Klebsiella pneumoniae* and *Citrobacter freeundii* were isolated from 1 (0.8%), 1 (0.8%) and 4 (3.3%) of the 123 samples, respectively. The *bla*KPC,gene was detected by PCR analysis in the *E. coli* and *K. pneumoniae* and all *C.* f*reeundii* isolates. Interestingly, one of the *C.* f*reeundii* isolates was also positive for the PCR of the VIM gene.

**Conclusions:** The spread of carbapenemase represents a serious threat to public health, for which it is important to investigate the sources and routes of transmission. Antimicrobial resistance monitoring programs in the EU focus on terrestrial animals. Bivalves are good indicators of environmental contamination and may act as possible carriers of bacteria derived from fecal pollution. Studies on the presence of carbapenemase-producing bacteria in bivalves are limited (3, 4, 5, 6). In this work, we report the presence of CPE in bivalves collected in Italy between 2024 and 2025 at sampling points in production areas of the central Adriatic Sea. Of these, *E. coli* and *K. pneumoniae* occurred in 0.8% of the analysed samples, while *C. freeundii* was more frequently isolated (3.3%). Carbapenemase-production in CPE from clams was determined by the presence of a KPC gene, one of the most prevalent enzymes conferring carbapenem-resistance in Gram-negative bacteria. Interestingly, in one of the *C.* f*reeundii* isolates was also detected the presence of the VIM gene. Characterisation of antimicrobial resistance gene carriage of CPE from clams by whole genome sequencing is ongoing.

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## P-043

# PCR screening for common carbapenemase genes in *K. pneumoniae* isolated from wastewater samples

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**Background and objectives:** Infectious diseases and the associated antimicrobial resistance (AMR) represent a global threat to human health. Carbapenemase-producing *Enterobacteriaceae* are listed as critically important pathogenic bacteria by the World Health Organization (WHO). One representative is *Klebsiella pneumoniae*, a species that is known for its increasing resistance to different antimicrobial agents. The detection of carbapenemase-producing *K. pneumoniae* in wastewater samples is significant for both wastewater-based epidemiology (WBE) and for investigating wastewater as a reservoir for the transmission of AMR into the aquatic environment.

The aim of this study was the detection and confirmation of carbapenemase production in K. pneumoniae isolated from wastewater samples.

**Methods:** Therefore, K. pneumoniae was isolated from the influent of four sewage treatment plants in Mecklenburg-Vorpommern, Germany, from July to November 2024, and investigated using antibiotic susceptibility testing (AST).

**Results:** A total of 259 isolates were phenotypically tested for carbapenemase production and subsequently genetically screened regarding frequent carbapenemase genes (i.e. $bla_{OXA-48}$, $bla_{NDM-1}$,

$bla_{KPC}$, $bla_{VIM}$, and $bla_{IMP}$) by PCR. As a result, the $bla_{OXA-48}$ gene was detected most frequently (n=125), followed by $bla_{NDM-1}$ (n=99) and $bla_{KPC}$ (n=53). There were differences in the distribution and combination of the genes tested across the distinct WWTPs. $Bla_{OXA-48}$ and $bla_{NDM-1}$were detected in all treatment plants, albeit in varying proportions, while $bla_{KPC}$ was predominantly detected in one plant.

**Conclusions:** Thus, this study was able to detect carbapenemase-producing *K. pneumoniae* in wastewater samples.

## P-044

# Whole-genome sequencing reveals high-risk clones of *Pseudomonas aeruginosa* in companion animal isolates from Germany

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**Background and objectives:** *Pseudomonas* (*P.*) *aeruginosa* is a pathogen of critical importance to One-Health as it plays a significant role in nosocomial infections and zoonotic transmission. Certain high-risk clones (HRCs) of *P. aeruginosa* are spread worldwide, and show increased virulence, resistance to multiple antimicrobial agents, and have epidemic potential. While extensive research has documented the impact of these HRCs in human healthcare settings, the prevalence and genetic characteristics of these strains in companion animals remain largely unknown. This study seeks to fill that gap by identifying and characterizing HRCs in *P. aeruginosa* isolates from companion animals in Germany.

**Methods:** A total of 72 *P. aeruginosa* isolates were collected from companion animals in Germany, which included dogs (n=58), cats (n=11), horses (n=2), and rabbits (n=1). Whole-genome sequencing and multilocus sequence typing were conducted to determine the sequence types (STs) and identify HRCs. The genomic analysis encompassed screening for virulence factors, resistance genes, and phylogenetic relationships.

**Results:** A comprehensive genomic analysis of 72 isolates from companion animals in Germany identified five STs (ST244, ST253, ST277, ST308, ST395), which belong to globally recognized HRCs. These HRCs were identified in isolates from dogs (n=7) and cats (n=4), obtained from various anatomical sites, including the ear, vagina, nose, skin, and wounds.

**Conclusions:** This study offers genomic insights into high-risk *P. aeruginosa* clones found in companion animals in Germany, indicating their possible potential as reservoirs for antimicrobial resistance. The findings demonstrate the need for ongoing surveillance and effective antimicrobial stewardship in veterinary settings to help prevent the spread of resistant pathogens across different species and environments.

**P-046**

# Too Much, Too Soon? Antibiotic Use, Resistance and Clonal Lineages of Uropathogenic *Escherichia coli* in Dogs and Cats

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**Background and objectives:** Urinary tract infections (UTI), which are frequently caused by *Escherichia (E.) coli,* are a common reason for antimicrobial use in companion animals and may promote antimicrobial resistance (AMR). While veterinary guidelines recommend amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), or trimethoprim-sulfamethoxazole (SXT) for empirical therapy, second-line antibiotics like fluoroquinolones (FQ) and 3rd generation cephalosporins (3GC) - classified by the WHO as Highest Priority Critically Important Antimicrobials (HPCIA) - are still frequently prescribed. We evaluated antibiotic therapy of UTI in relation to treatment guidelines, explored phenotypic and genotypic resistance profiles of uropathogenic *E. coli* (UPEC) and the putative presence of dominant clonal lineages among UPEC from dogs and cats.

**Methods:** Between November 2019 and November 2020, 1,862 urine samples from dogs and cats were analysed. Questionnaire data on prior antimicrobial treatment were available for 113 dogs and 48 cats. In total, 343 *E. coli* isolates (221 canine, 122 feline) underwent antimicrobial susceptibility testing (Micronaut, Bruker) and whole genome sequencing. *In silico* analysis included detection of resistance and virulence genes, multilocus sequence typing (MLST), and core genome MLST (cgMLST; Ridom SeqSphere+ v10.0.5).

**Results:** *E. coli* was the isolated pathogen in 47.6%/50.2% of dogs/cats. AMC was the most frequently prescribed antibiotic (63.2%/34.6%), while AMX (4.4%/15.4%) and SXT (4.4%, dogs only) were rarely used. Notably, FQs like enrofloxacin (ENR) and pradofloxacin (PRA) as well as the 3GC cefovecin (CFV) were used in 20.6%/46.1%. Phenotypic resistance (CLSI VET01S ED7) rates were: AMP (22.2%), SXT (11.7%), AMC (5.0%), ENR (9.3%), PRA (7.9%), and CFV (3.5%). Multidrug resistance was determined in 18.3% of the isolates. CFV resistance was significantly associated with the presence of ESBL and AmpC β-lactamase genes (5 x *bla*$_{\text{CTX-M-15}}$; *bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-27}}$, *bla*$_{\text{DHA-1}}$, *bla*$_{\text{CMY-2}}$, 1 x each), and chromosomal mutation ampC_C-42T ($p$<0.001). One ST372 isolate from a cat harboured *bla*$_{\text{OXA-48}}$.

ST73, which is one of the most dominant UPEC clones in humans, and ST372 were most prevalent (16.6% each). ST372 dominated in dogs (24.9%), ST73 in cats (27.1%). The two dominant clonal complexes, CC73 and CC372, differed markedly: CC73 showed high genetic diversity, with multiple subclusters and single locus variants whereas CC372 was much more homogeneous. Both CCs showed low resistance levels to first-line and full susceptibility to second-line antibiotics. In contrast, the other most common STs (ST12, 9.3%; ST127 and ST141, 4.4% each; ST131, 3.2%) showed resistance to CFV (2.7%), ENR (4.1%), and PRA (2.7%).

**Conclusions:** *E. coli*, the most common UTI pathogen in companion animals, showed low AMR to first- and second-line antibiotics, indicating that the frequent empirical use of HPCIA was not justified. ST372 and ST73 appeared as dominant, but largely susceptible lineages, differing in host association and genomic diversity.

**P-047**

# Monitoring of the Phylogenetic Heritage and Antimicrobial Resistance Characteristics of *Klebsiella pneumoniae* Isolated in a Veterinary Diagnostic Laboratory in 2022-23

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**Background and objectives:** *Klebsiella* (*K.*) *pneumoniae* is an opportunistic pathogen of the order *Enterobacterales* that is widely associated with antimicrobial-resistant (AMR) infections in humans and animals worldwide, such as bloodstream, respiratory and wound infections. Multidrug resistance (MDR) and the production of extended-spectrum β-lactamases (ESBL), AmpC β-lactamases and carbapenemases pose a particular threat to public health due to limited treatment options. The emergence of high-risk *K. pneumoniae* clones, such as ST307, and the successful dissemination of transferable resistance plasmids are key drivers in the global spread of AMR.

**Methods:** In 2022/2023, 202 *K. pneumoniae* were isolated in the Institute"s diagnostic laboratory from clinical samples of companion animals (81.2%), livestock (15.8%) and exotic animals (3.0%), received from 72 veterinary institutions. For species identification, MALDI-TOF MS (DB 9045, Bruker Daltonics) was employed. Antimicrobial susceptibility testing against substances used in veterinary and human medicine was performed by broth microdilution (Micronaut-S v.6.00, Bruker Daltonics; VITEK®2 compact v9.03.3, bioMérieux). Whole genome sequences were analysed *in silico* for multilocus sequence types (STs), AMR determinants and plasmid types, using ResFinder v.4.6.0, Ridom v.10.0.5, Geneious v.2023.2.1, and BIGSdb v.1.50.2.

**Results:** An MDR phenotype (i.e. 4.0% carbapenem, 8.4% tigecycline, 2% colistin and 14.4% fluoroquinolone non-susceptibility) was found in 85 isolates (42.1%), mainly from companion animals (58.7%; dog 46.8%; cat 11.9%; $p < 0.001$). The carbapenemase gene *bla*$_{\text{OXA-48}}$ (3.0%) was detected in six dogs, while the AmpC gene *bla*$_{\text{DHA-1}}$ (1.5%) was found in three isolates originating from the same dog. ESBL genes (6.9%; *bla*$_{\text{CTX-M-15}}$, n = 13; *bla*$_{\text{CTX-M-3}}$, n = 1) and several other AMR genes were detected in various animal species. Regarding resistance plasmids, *bla*$_{\text{OXA-48}}$ was mainly (83.3%) located on an IncL plasmid (approx. 63 kbp), 78.6% of ESBL genes were located on an IncFIB/IncFII plasmid (approx. 211 kbp) and all *bla*$_{\text{DHA-1}}$ were located on an IncR plasmid (approx. 51 kbp). In total, isolates belonged to 143 different STs (Shannon index: 4.8), indicating very high diversity. Sequence types included MDR high-risk clones frequently isolated in both humans and animals (ST11, ST15, ST307). All ST147 isolates exhibited an MDR phenotype (n = 8, $p < 0.001$) and originated from the same clinic. A significant number of *bla*$_{\text{OXA-48}}$-harbouring isolates belonged to ST1248 (n = 3, $p < 0.001$), also originating from the same clinic.

**Conclusions:** We observed a generally high phylogenetic diversity among animal *K. pneumoniae* isolates, with a cluster of the well-established human MDR ST147. Moreover, the prevalence of plasmid-located AMR genes conferring resistance to both veterinary and human last resort antibiotics emphasizes the potential threat posed by such strains. Especially in light of the emergence of convergent *K. pneumoniae* carrying plasmids that confer not only MDR but also a hypervirulent phenotype.

**P-048**

# High proportions of Enterobacterales resistant to extended-spectrum cephalosporins in meat samples in Tunisia

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**Background and objectives:** Food-producing animals are potential reservoirs of resistances to antibiotics classified as critically important for human health, such as extended-spectrum cephalosprins (ESC) and carbapenems (CP). Even though human-to-human contacts are the primary vector of antimicrobial resistance (AMR) dissemination in the community, food consumption and preparation plays a non-negligible role in the global burden of AMR. The goal of this study was thus to investigate the occurrence of ESC- and CP-R Enterobacterales in different types of meat in Tunisia.

**Methods:** In 2024, 71 samples of meat products (chicken, n=25; sheep, n=30; bovine, n=7; goat, n=6; camel, n=3) were collected. After enrichment, 100 ml of overnight cultures were inoculated on MacConkey agar plates supplemented with cefotaxime (2µg/ml) or imipenem (2µg/ml). Antimicrobial susceptibility was tested by disc diffusion and all resistant isolates were short-read sequenced (Illumina). After quality check, the presence of resistance genes was detected, together with the plasmid content. A cgMLST-based phylogeny was performed. Subsets of isolates were further long-read sequencing to characterize resistance-carrying plasmids.

**Results:** A high proportion (46/71, 64.8%) of meat samples were contaminated with ESC-R Enterobacterales, but no CP-R isolate was identified. Chicken meat was the most heavily contaminated one (80% of samples). Identification showed the presence of *E. coli* (34/50, 68%) *Klebsiella pneumoniae* (26%) and *Enterobacter hormaechei* (6%), which belonged to a high diversity of clones (15 different STs for *E. coli*, seven for *K. pneumoniae*). Among the six *E. coli* and three *K. pneumoniae* STs that were found in more than one isolates, clonal dissemination was recurrently observed. The ESC phenotype was due to extended-spectrum beta-lactamases (ESBLs), with the identification of the *bla*CTX-M-15 (n=30), *bla*CTX-M-55 (n=12), *bla*CTX-M-1 (n=3) and *bla*SHV-12 (n=1) genes. All *bla*CTX-M-55 genes were carried on the chromosome, while *bla*CTX-M-15 genes were found on IncF plasmids, among which 10 belonged to the IncF/F-:A-:B53 formula. Finally, one *bla*CTX-M-1 gene was carried by an IncI1/ST3 plasmid; the two others were carried by an IncHI2/ST4 plasmid co-harbouring the *mcr-1* gene.

**Conclusions:** Characterization of the collected isolates showed an important contamination (64.8%) with ESC-R Enterobacterales, disseminated by a large diversity of clones, genetic determinants and resistance genes, suggesting complex dynamics of emergence, spread and persistence in this food sector. Also, a few clones (ST2973, ST155, ST117) and plasmids (IncF/F-:A-:B53, IncY) were identified, that have both a zoonotic importance and a One Health relevance. Finally, this study highlighted the need of improving control and prevention strategies in order to decrease the AMR burden in Tunisia.

**P-049**

# Prevalence of carbapenemase-producing Enterobacterales (CPE), extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, methicillin-resistant *Staphylococcus aureus* (MRSA), and plasmid-mediated colistin resistance in Enterobacterales in food-producing animals in Suriname

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**Background and objectives:** Suriname, a developing country situated on the north coast of South America, relies on both imports and domestic production for its beef, pork, and poultry supply. Food products and direct contact with animals may transmit antimicrobial-resistant bacteria to humans. There is a lack of knowledge on the prevalence of antimicrobial-resistant bacteria in food-producing animals in Suriname. As part of a larger One Health research project, the main objective of this study was to investigate the prevalence of antimicrobial resistance carriage in cattle, pigs, and poultry in Suriname. We focused on specific highly resistant microorganisms, including carbapenemase-producing Enterobacterales (CPE), extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales, methicillin-resistant *Staphylococcus aureus* (MRSA), and plasmid-mediated colistin resistance in Enterobacterales. Furthermore, we assessed the susceptibility of the indicator organism *E. coli* to a panel of antimicrobials across all three animal species.

**Methods:** Sampling in Suriname was conducted from November 2023 to January 2025; pooled faecal samples were collected from pigs and poultry, while individual faecal samples were obtained from cattle. Samples were stored at -20°C until analysis by culture-based methods to detect the presence of carbapenem-resistant and ESBL-producing Enterobacterales, and MRSA. To detect plasmid-mediated colistin resistance, the samples were screened for mcr-1-10 genes by PCR. Indicator *E. coli* isolates were tested for antimicrobial susceptibility to a fixed panel of antibiotics using broth microdilution, according to the Dutch national surveillance protocol. All resistant bacteria isolated were sequenced on an Illumina platform.

**Results: Table 1** shows the highly resistant microorganisms we found in faecal samples from cattle, pigs and poultry.

Table 1. Highly resistant microorganisms found in faecal samples from cattle, pigs and poultry.

| | N samples | N suppliers | CPE | ESBL | MRSA | Plasmid-mediated colistin resistance |
|---|---|---|---|---|---|---|
| **Cattle** | 111 | 47 | 1 in Enterobacter spp. | 2 in *K. pneumoniae* (4.3% of the suppliers) | None | None |
| **Pigs** | 111 | 20 | None | 39 in *E. coli*(76.2% of the suppliers) | 22 (46.6% of the suppliers) | 6 mcr-1 in *E. coli* (20% of the suppliers) |
| **Poultry** | 111 | 24 | *Expected* | *Expected* | *Expected* | *Expected* |

The Enterobacter spp. carried a *bla*IMI-1 -gene; various ESBL-genes were detected in the ESBL-positive isolates and all MRSA were of the ST 398 and carried *mecA*.

The indicator *E. coli* from pigs and cattle showed low resistance profiles. In pigs, the highest resistance percentages were found for tetracycline (23.1%), sulfamethoxazole (13.0%), and ampicillin (9.3%). Twenty-two out of the 69 resistant isolates were resistant to two or more antimicrobials. In cattle, one isolate was resistant only to tetracycline, one isolate was resistant to ciprofloxacin and nalidixic acid and one isolate was resistant to eight antimicrobials.

**Conclusions:** This study demonstrates that highly resistant microorganisms are frequently identified in food-producing animals in Suriname, with prevalence up to 76% among pig suppliers. A greater abundance and diversity of resistant bacteria was observed in pigs compared to cattle, which may be due to the more intensive husbandry practices employed in the pig industry. The presence of resistant bacteria in livestock signifies a potential threat to public health. Additional research is crucial to evaluate the risk of transmission to humans and its effects on public health.

## P-051

# Dynamics of emerging antibiotic resistance genes of public health concern in the Montpellier aquatic environment: toward a genomic proxy for risk exposure

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**Background and objectives:** A multisectoral "One Health" approach is now recommended to study and combat antimicrobial resistance (AMR), a growing public health concern. The environment serves as both a reservoir and a transmission route for AMR, with water systems facilitating interactions and antimicrobial-resistant gene (ARG) transmission between antimicrobial-resistant bacteria and sensitive bacteria. Mobile genetic elements (MGEs) are known to play a crucial role in microbial ecology by facilitating the horizontal transfer of genes across microbial populations. However, genomic criteria for

assessing the risk of ARG enrichment in aquatic environments are still lacking within the global AMR cycle.

**Methods:** We address this gap at the local scale by establishing a collection of 300 carbapenem-resistant strains from diverse points of a well-characterized watershed, the Lez River basin: Lez spring water, hospital wastewater, surface water in an urban river near the hospital, wastewater treatment plants, and patient samples from Montpellier hospitals. Whole-genome sequencing of all strains was performed using long-read sequencing. De novo assembly and comprehensive analysis of the mobilome and resistome were conducted using ResMobiLys (publication in preparation). Combining environmental and clinical samples allows to address shared and specific resistome and mobilome features of the different environments and infer how resistances propagate through environments.

**Results:** Efflux pumps are predominant and represent the main resistance mechanism. The prevalence of beta-lactamase genes is higher in wastewater treatment plants, hospital wastewater, and patient samples. In these environments, beta-lactamase genes are 3–4 times more abundant than in spring water and surface water rivers near the hospital (and twice as high for efflux pumps). Carbapenemase genes were identified in all environments, such as OXA-48 in Lez spring water. MGE abundance was significantly higher in patient samples, hospital wastewater, and wastewater treatment plant compared to spring water and surface water rivers near the hospital, suggesting an increased potential for ARG mobility. Shared resistance-carrying plasmids, including carbapenemase plasmid, were identified in Enterobacter and Aeromonas isolates both within and between environments, making them good candidates for resistance propagation. These bacteria could act as intermediaries or shuttles contributing to ARG dissemination.

**Conclusions:** These initial and partial results underline the complex implication of aquatic bacterial communities in the environmental AMR-cycle, which is largely neglected. Ultimately, this study will help identify genomic features and key points in the cycle of ARG across aquatic environments, providing targets for surveillance and strategies to reduce the environmental reservoir of AMR.

## P-052

# Phylogeny, antimicrobial resistance genes and virulence factors differ in populations of *Escherichia coli* and cefotaxime-resistant *E. coli* isolated in pairs from composite fecal samples of fattening pigs at a German slaughterhouse

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**Background and objectives:** *Escherichia coli* inhabits the vertebrate gastrointestinal tract as a commensal, but its presence can also cause a variety of diseases, with antimicrobial-resistant pathovars representing a serious danger to human and animal health. While cephalosporin-resistant strains have been well-characterized globally, less is known about resistant commensal strains and how they interconvert. We, therefore, investigated corresponding pairs of *E. coli*, selected from plates without an antibiotic, and cefotaxime-resistant *E. coli* (CTX-*E. coli*), isolated from 398 composite porcine fecal samples taken at a German slaughterhouse, for their population structure, antimicrobial resistance genes and virulence factors.

**Methods:** Whole-genome sequencing of 398 corresponding *E. coli* and CTX-*E. coli* isolate pairs was performed on the Illumina platform, followed by assembly and sequence analysis with, e.g., multilocus sequence typing [1], ResFinder [2] and VirulenceFinder [3].

**Results:** The *E. coli* and the CTX-*E. coli* populations differed strongly in their phylogeny and in their sequence types (ST). CTX-*E. coli* were more strongly present in phylogroups C and G, they were

roughly equally present in phylogroup B1 and they were less frequently present in phylogroup A. With 119 different STs [1], the *E. coli* population was more diverse than the CTX-*E. coli* population with 90 STs. For STs containing five and more members, five (10, 23, 58, 88, 101) were present in both populations, one (4519) was unique among CTX-*E. coli* and three (542, 898, 3057) in the *E. coli* population. One of the latter, ST542, had 27 members and was the second most frequent ST in the *E. coli* population. Among the 15 STs in the CTX-*E. coli* population with five and more members, seven (10, 23, 58, 88, 117, 131, 410) were classified as belonging to the top 20 STs associated with extraintestinal pathogenic *E. coli* (EXPEC) [4]. In contrast, in the corresponding *E. coli* population, only four of 14 STs (10, 23, 58, 88) were associated with an ExPEC phenotype. Acquired antimicrobial resistance genes were roughly twice as frequent in the CTX-*E. coli* isolates (6.07±3.01) than in the *E. coli* population (3.17 ± 2.68). This is also the case for virulence factors with mean values of 15.62 ± 7.14 vs. 8.32 ± 6.85. Further analyses addressing plasmids, mobile genetic elements and selected sequence types are ongoing.

**Conclusions:** Both populations differed in phylogeny and their antimicrobial resistance gene and virulence factor counts. CTX-*E. coli* strains bore STs more frequently associated with an ExPEC pathotype. Consequently, surveillance targeting CTX-*E. coli* remains important, as these apparently pose greater risk to public health.

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## P-053

# Comparative genomics of *Escherichia coli* from urinary tract infection and bacteraemia isolates in the Czech Republic

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**Background and objectives:** Extraintestinal pathogenic *Escherichia coli* (ExPEC) strains pose a significant burden on global healthcare systems. Dissemination of multi-drug resistant ExPEC lineages was repeatedly documented but the role antibiotic-susceptible lineages still remain underreported. We aim to provide a comprehensive study of both susceptible and antibiotic resistant ExPEC isolates, with the emphasis on their susceptibility to critically important cephalosporins and emerging lineages.

**Methods:** A total of 627 *E. coli* isolates were collected between 2022 and 2023 along with clinical data from 10 Czech hospitals. Isolates were obtained from patients with urinary tract infection (n=347) and bacteriemia (n=280) and were collected from community (n=248), hospitals (n=366) or information was not provided (n=13). Equal number of isolates susceptible (CTX-S, n=355) or resistant (CTX-R, n=272) to cefotaxime were collected. Susceptibility to a set of antibiotics using minimal inhibition concentration, whole-genome sequencing, comparative genomics and other bioinformatical and statistical analyses were performed.

**Results:** ExPEC isolates were significantly more prevalent among women and older patients and hospital isolates were significantly more resistant than community isolates. In susceptible population predominated *bla*TEM-1B resistance gene; among resistant population predominated *bla*CTX-M-15. The differences in antibiotic resistance or presence of virulence-associated genes (VAGs) between isolates were usually ST-dependent without significant relation to the sample origin (urine, blood). CTX-R isolates carried significantly less VAGs than CTX-S ones. The most prevalent sequence types (ST) isolated as CTX-S consisted of ST73 (14%), ST95 (13%); primarily CTX-R lineage was ST131 (52%). ST69 was frequently present in both CTX-S (19%) and CTX-R (8%) groups. No differences were observed between blood and urine isolates in terms of ST distribution. The most prevalent virulence plasmids were ColV (27%) which was predominantly in ST69 and ST95 and pUTI89 F29:A-:B10 (12%) which was associated mainly with ST131, ST404, ST69 and ST73.

**Conclusions:** Our study highlights differences in the genomic characteristics of CTX-S and CTX-R ExPEC isolates. The frequent presence of virulence plasmids in ExPEC, such as the common association of pUTI and ST131, underscores the potential risk of horizontal transfer of virulence gene. This study emphasizes the need for continued surveillance of both resistant and susceptible ExPEC.

## P-054

# Carbapenem resistant Enterobacterales infections and environmental shedding in a veterinary teaching hospital

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**Background and objectives:** Background: Carbapenem resistant Enterobacterales (CREs) are emerging pathogens causing nosocomial infections in humans. In veterinary medicine, data is limited.

Objectives: to determine the prevalence of CRE infections and environmental contamination in a tertiary veterinary hospital.

**Methods:** Methods: Clinical isolates (February 2020-February 2021) from the Koret School of Veterinary medicine- Veterinary Teaching Hospital were screened for imipenem resistance. Additionally, 100 environmental samples were collected from surfaces and medical devices, using sterile sponges. Imipenem resistance was confirmed via disc diffusion testing and Vitek-2 analysis (human card N395 and veterinary card GN98). Lateral flow assays were employed to identify resistance genes.

**Results:** Results: of 775 bacterial isolates, 12 isolates (1.5%), originated from five dogs and four cats (one cat provided four samples), were resistant to carbapenems. Bacterial species were multidrug resistant, including 11 *Escherichia coli* isolates and one *Proteus mirabilis* isolate. Samples origins included urine (58.3%), abdominal cavity (25%) and wounds (16.7%). The predominant carbapenemase were NMD (10 *E. coli* isolates) and OXA (1 *E. coli*). Three of 100 environmental samples tested positive for CRE, yielding two *Enterobacter* spp. and one *E. coli* isolate, harboring the NMD gene. Out of nine CRE infected animals, three died; two suffered from urinary tract infection and one from septic peritonitis.

**Conclusions:** Conclusions: Carbapenem-resistant Enterobacterales are significant emerging pathogens in veterinary clinical infections and pose a contamination risk within veterinary hospital environments. These findings underscore the urgent need for robust antibiotic stewardship and enhanced infection control measures.

**P-055**

# Antimicrobial-resistant bacteria in marine mammals along the Japanese coast

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**Background and objectives:** Antimicrobial-resistant bacteria are widely found in the ocean and marine mammals; however, their distribution in wild marine mammals remains understudied in Japan [1,2]. Here, we aim to investigate the presence and distribution of antimicrobial-resistant bacteria in stranded finless porpoises and dolphins.

**Methods:** Stranded finless porpoises and dolphins were collected from Japan"s coastal areas between 2022 and 2024. Gut contents were sampled and preserved in 20% glycerol and stored at -80°C until use. Samples were cultured on sheep-blood agar and Mac Conkey agar and incubated at 37°C for 18-24 hours. Colonies with distinct morphologies were isolated, and species identification was performed using MALDI-TOF (Shimadzu) or 16s rRNA gene sequencing. Antimicrobial-susceptibility was tested using Vitek®2 Compact system (Biomerieux) according to CLSI standards. Whole-genome sequencing of selected isolates was conducted on Illumina MiSeq platform followed by assembly and sequence analysis.

**Results:** A total of 59 finless porpoises and dolphins were collected from coastal regions in Japan such as Nagasaki, Oita, and Ehime. Various species of *Enterobacteriaceae* and Enterococcus were isolated, which includes *Escherichia coli, Acinetobacter junii, Acinetobacter baumanii, Alcaligenes faecalis, Enterobacter sp., Klebsiella oxytoca*, and others. Multiple-drug resistant (MDR) bacteria were identified in *Alcaligenes faecalis*, *Escherichia coli*, and *Enterobacter cloacae*. One *E. coli* isolate from a striped dolphin exhibited MDR characteristics, displaying resistance to ampicillin, amoxicillin/clavulanic acid, piperacillin, and multiple cephalosporins, including cefazolin, cefoxitin, cefotaxime, cefotiam, others. This strain was identified as an extended-spectrum beta-lactamase (ESBL), MDR genes are currently under investigation, and the responsible genes will be presented at the conference.

**Conclusions:** Enterobacteriaceae species commonly found in terrestrial mammals were also isolated from stranded marine mammals in Japan. Many of these bacteria exhibited resistance to multiple antibiotics, suggesting potential that these bacteria may have derived from human activities. Further genomic analysis and environmental studies of coastal areas are necessary to assess the impact of human activities on marine mammal microbiomes.

**P-056**

# Multi-susceptibility – a validated One Health indicator of antimicrobial resistance

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**Background and objectives:** Conducting integrated analyses on antimicrobial resistance from a One Health perspective and communicating on their results may come up against a diversity of bacterial-antibiotic pairs that could be studied [1]. Defining a synthetic indicator common to humans and animals could be a first step towards a joint approach and communication on antimicrobial resistance. A "complete susceptibility" indicator has been proposed as a key indicator in healthy slaughtered animals [2]. Extension of this indicator is not possible in absence of harmonisation across monitoring systems. Our objective was to define a relevant indicator that could be estimated by all antimicrobial resistance monitoring systems existing in France.

**Methods:** An indicator of *Escherichia coli* multi-susceptibility was firstly jointly defined by members of the surveillance study group of the French professional community network PROMISE. Data were collected from the different monitoring programs, according to the combination of the common set of antimicrobials, over a ten years period, at the national and regional level, in both humans (from hospitals, community or nursing home) and animals (healthy and diseased animals from different species). A validation process was secondly applied to determine indicators" properties, interest and relevance through the assessment of objective criterions [3, 4].

**Results:** It was possible to calculate a percentage of *E. coli* isolates susceptible to five antimicrobial classes tested in both humans and animals, in all species or populations. Moreover, the indicator was found to i) be estimated on a large unbiased set of isolates (i.e. the five antimicrobial classes were tested for a large amount of isolates), ii) vary across regions and populations and iii) show trends (making its monitoring relevant) iv) be strongly and significantly related to antimicrobial use in all populations and species (allowing evaluation of interventions) v) not be redundant with other indicators already implemented.

**Conclusions:** The common set of antimicrobial classes addressed by the "multi-susceptibility" indicator is incomplete and potentially perfectible. The indicator could nevertheless be validated. Indicator validation is a rarely used process, mainly developed in ecological studies, a framework appropriate to antimicrobial resistance in a One Health perspective. A criterion: the satisfactory appropriation by users, remains to be assessed, as the extension of the indicator to the environment.

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**P-057**

# Global Perspective on Antimicrobial Use and Resistance in Poultry

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**Background and objectives:** The increase in antibiotic resistance is a global concern for human and animal health. Poultry is one of the world´s fastest growing sources of meat production. This study identifies the antibiotics legalized and the levels of antibiotic resistance reported in Escherichia coli isolated from broilers originating from large poultry-producing regions, including the US, China, Brazil and countries of the EU (Poland, United Kingdom, Germany, France and Spain), which produce more than half of the global poultry meat supply. The objective of this review was to identify the type and amount of antibiotics used in poultry production and the level of antibiotic resistance in *E. coli* isolated from broilers.

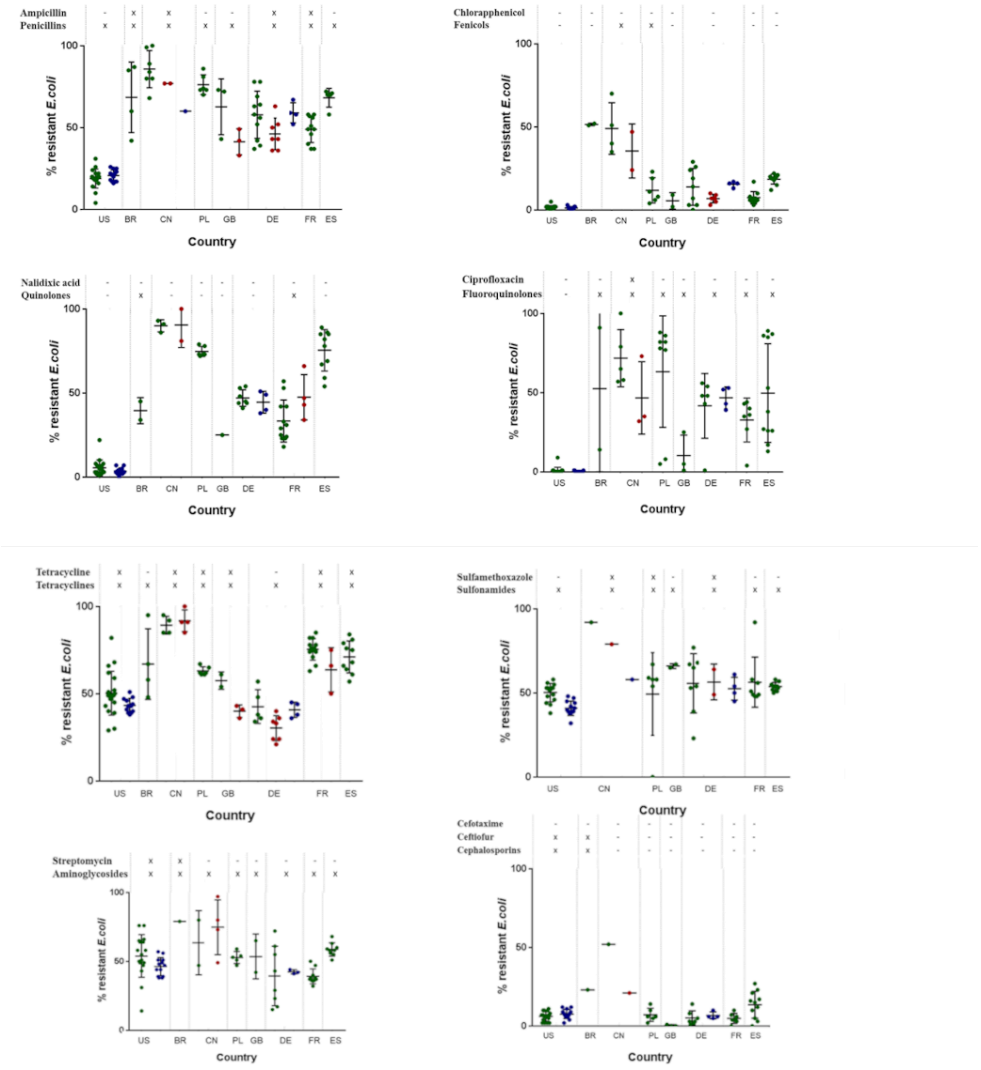
**Methods:** Publicly available data published by governmental authorities were used to examine antibiotics approved and banned for use in poultry production in the United States, China, Brazil, Poland, the United Kingdom, Germany, France, and Spain. These data were combined with antimicrobial resistance data from national and international monitoring programs, as well as scientific publications, to provide a comprehensive global overview of available information on antimicrobial use and resistance in poultry production.

**Results:** There is no public long-term quantitative data available on the amount of antibiotics used in poultry, with the exception of France. Qualitative data of registered antibiotics enabled their evaluation. Data on antibiotic resistant *E. coli* is available for most regions but detection of resistance and number of isolates in each study differs among regions, therefore statistical evaluation was not possible.

Data from France indicate that the decreased use of tetracyclines leads to a reduction in the detected resistance rates. The fluoroquinolones, 3rd generation cephalosporins and macrolides are approved for use in large poultry-producing regions, with the exception of fluoroquinolones in US and cephalosporins in the EU. The resistance rates to fluoroquinolones in the US, where fluoroquinolones are not registered for use, are below 5%, while the average of resistant *E. coli* is above 40% in Brazil, China and EU, where use of fluoroquinolones is legalized. Tetracyclines, aminoglycosides, sulfonamides and penicillins are registered for use in poultry in all evaluated countries. The average resistance rates in *E. coli* to representatives of these antibiotic classes are higher than 40% in all countries, with the exception of ampicillin in the US.

**Conclusions:** Globally available data on antibiotic use and resistance clearly demonstrate a direct link between antibiotic application in broiler production and the emergence of antibiotic resistance. Furthermore, the data reveal that Highest Priority Critically Important Antimicrobials (HPCIAs) are approved for use in broilers in major poultry-producing countries.

**Figure**




**P-058**

# High throughput qPCR analyses suggest that Enterobacterales of French sheep and cow cheese rarely carry genes conferring resistances to critically important antibiotics for human medicine

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**Background and objectives:** Bacteria present in raw milk can carry acquired or intrinsic antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs). However, only a few studies have evaluated raw milk cheese as a potential reservoir of ARGs. This study thus aimed at providing new data regarding resistance markers present in raw milk cheese.

**Methods:** Sheep (n=360) and cow (n=360) cheese samples produced in France were incubated in buffered peptone water supplemented with acriflavin or novobiocin and total DNA was extracted. High-throughput microfluidic real-time PCR amplification of 30 ARGs and 16 MGEs associated to gram-negative bacteria was performed using 48.48 dynamic arrays (Biomark™; Standard Biotools, USA) on these total DNA extracts after a pre-amplification step.

**Results:** As corroborated by 16S metabarcoding, samples were enriched in Gram-negative bacteria since *Escherichia coli* and *Hafnia alvei* respectively accounted for 40% and 20% of the samples" microbiota. Screening of the samples for the presence of 30 ARGs and 16 MGEs by high throughput qPCR array showed that nine ARGs conferring resistances to 1st-generation beta-lactams, aminoglycosides, trimethoprim/sulfonamides and tetracyclines occurred in more than 75% of both sheep and cow samples. This is neither surprising nor alarming since these resistance genes are widely spread across the One Health human, animal and environmental sectors. Conversely, genes conferring resistances to last-generations cephalosporins were rarely identified, while those conferring resistances to carbapenems or amikacin, which are restricted to human use, were never detected. Multiple MGEs were detected, the most frequent ones being IncF plasmids, confirming the potential transmission of ARGs.

**Conclusions:** Our results are in line with the few studies of the resistome of milk or milk cheese showing that genes conferring resistances to 1st-generation beta-lactams, aminoglycosides and tetracyclines families are widespread, while those conferring resistances to critically important antibiotics are rare or absent.

## P-060

# Improving the use of class 1 integrons as a proxy for antibiotic resistance in the environment

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**Background and objectives:** Quantitative PCR (qPCR) is an efficient and relatively inexpensive approach to assess the abundance of antibiotic resistance markers in the environment. However, the selection of the panel of a few antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) to be targeted is still a matter of debate. The integrase gene (*intI1*) of the class 1 integrons is probably the only consensual marker to monitor, as its abundance correlates with those of ARGs/MGEs in environments affected by human activities. However, this is not always the case in pristine-like environments, making the use of this proxy imperfect. We here propose a simple method improving the use of this marker.

**Methods:** Artificial exposure units allowing the collection of biofilms were installed at 6 sites along the course of the Orne River for 3 weeks. This 85 km long river originates in a forest, then flows through an agricultural area, then through a highly urbanized and industrial area, before reaching its mouth in the Moselle. Six such sampling campaigns were carried out over 1 year (every 2 months). Total DNAs were extracted from the biofilms and were analyzed by 16S metabarcoding and by qPCRs targeting the *intI1* gene, the 3 gene cassettes *aadB*, *aacA4* and *aadB*, and the 16S rDNA.

**Results:** Expressing the relative abundances of ARGs/MGEs as the classical ratio copies/16S rDNA copies did not allow to reveal any anthropic impact in the Orne River. However, expressing the relative abundances of the 3 gene cassettes as copies/*intI1* gene copies allowed to reveal statistically significant gradients from upstream to downstream. These differences, in particular the fairly constant

*intI1* abundance along the river course, were probably due to the presence of more cassette-free integrons at the source of the river. The anthropogenic gradient was confirmed by the metabarcoding data and the increasing abundances of human-associated bacteria (*Bifidobacteria*) from the source to the mouth of the river. It was also highlighted by the relative abundances of the 3 gene cassettes expressed per *intI1* gene copies *i.e*. the cassette composition of the class 1 integrons showing that the bacterial communities from each sampling site have their own pattern reflecting the particular anthropogenic pressures at each sampling site. Determining the cassette composition of the class 1 integrons in water samples collected from 3 French rivers and 3 municipal and 1 slaughterhouse wastewater treatment plants has shown that the bacterial communities of each of these environments have their own pattern.

**Conclusions:** The easiness and robustness of the qPCR approach here presented have led the French meta-network PROMISE gathering people interested in antibiotic resistance at the national level to use it as a standardized method to be implemented while studying and monitoring environmental samples.

## P-061

# Poultry Production Systems as Reservoirs of Sulphamethoxazole and Ampicillin Resistance in Sierra Leone

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**Background and objectives:** Between 2019-2023, Sierra Leone has seen a rise in the number of commercial poultry farms. Some poultry farmers in Sierra Leone use antibiotics for prophylactic, therapeutic, and growth promotion uses often without veterinary prescription. Studies have shown drugs used by farmers on livestock in Sierra Leone are among the "watch" category of WHO AwaRe classification. This exposes consumers to antibiotic resistant gastrointestinal bacteria. The objective of this study was to determine the antibiotic susceptibility profiles of *Escherichia coli* and *Salmonella* spp. isolated from layer chickens in Sierra Leone. This type of study is the first, studying the burden of AMR in indicator *E. coli* and *Salmonella* in poultry from commercial poultry farms.

**Methods:** A total of 137 farms were sampled from 5 selected districts between August 2023 and February 2024. 5 cloacal swab samples were pooled representing each farm. For isolation, we cultured samples on MacConkey & Eosin Methylene Blue Agar for *E. coli* and Rappaport-Vassiliadis broth & Salmonella-Shigella Agar for *Salmonella*. All *E. coli* and *Salmonella* isolates were identified using API 20E Kit according to manufacturer"s instructions. Identified isolates were subjected to a panel of 12 antibiotics with varying antibiotic classes and zones of inhibition obtained were interpreted using CLSI guidelines.

**Results:** Of the 137 samples collected, 130 (94.9%) yielded *E. coli* while only 27 (19.7%) yielded *Salmonella* bacteria. The antibiogram showed that the *E. coli* isolates displayed the highest resistance to Sulphamethoxazole (90.8%) followed by Ampicillin (55.4%). In addition, 65 (50%) *E. coli* isolates had both Sulphamethoxazole and Ampicillin in their resistance profile. The antibiogram of *Salmonella* bacteria isolated revealed that the highest resistance was seen in Sulphamethoxazole (92.6%) followed by Ampicillin (81.5%). There was no resistance against carbapenems in both *E. coli* and *Salmonella* isolates. 46 (35.4%) and 19 (70.4%) of the *E. coli* and *Salmonella* isolates respectively were multi-drug resistant. Further, 47 (36.2%) and 19 (70.4%) of *E. coli* and *Salmonella* spp. isolates respectively had an MAR index greater than 0.2. Between 2023 (rainy season) to 2024 (dry season),

there was a decrease in resistance in 10 of the 12 antibiotics in *E. coli* isolates while in the *Salmonella* spp. there was a decrease in resistance against all 12 antibiotics.

**Conclusions:** The current study recorded a higher resistance rate by *E. coli* and *Salmonella* isolates against Sulphamethoxazole which is higher than the 62% recorded in the 2022 study of *E. coli* from poultry excreta used as fertilizers in Sierra Leone. The regular use of Sulphonamide based drugs for therapeutic and prophylactic purposes against coccidiosis by Poultry farmers, even in Sierra Leone, may be a contributing factor for this high resistance. More study is needed to study the antibiotic profiles of bacteria from poultry across different weather seasons in Sierra Leone. It may also be prudent to study the resistance genes circulating in bacteria in poultry systems in Sierra Leone. This study has filled an important evidence gap by providing the first assessment of resistance prevalence in indicator *E. coli* and *Salmonella* in Poultry farms in Sierra Leone. These data will inform development of future active AMR surveillance in Sierra Leone.

## *t02 - Mechanisms and dissemination of antimicrobial resistance in animal and zoonotic pathogens*

### P-063

## Critically important antimicrobial resistant *E. coli* from farm-to-fork in intensive broiler production in Pakistan

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**Background and objectives:** The emergence of resistance towards critically important antimicrobials in bacteria pose a serious threat to public health. We investigated the spread of last-resort critically important antimicrobials colistin and tigecycline resistance in *E. coli* in broiler production pyramid in Pakistan using genomics.

**Methods:** Integrated broiler production pyramid (farm-to-fork) sampling strategy was designed to collect AMR indicator *E. coli* from commercial broiler breeders, hatchery, day old and adult broiler birds and chicken meat in Pakistan. Whole genome sequencing was performed to determine the genomic features and mechanisms of resistance towards critically important antimicrobials colistin and tigecycline. Phenotypic antimicorbial resistance was determined using disc diffusion and broth microdilution methods.

**Results:** We demonstrated both clonal and vertical transmission of colistin and tigecycline resistant *E. coli* in broiler production pyramid in Pakistan. We found large diversity in sequence types of *E. coli* from broiler breeders, broiler chickens and chicken meat. However, *E. coli* ST1011 with few SNPs was present in broiler breeder, broiler chicken and meat. We found 12 *E. coli* carrying plasmid mediated tigecycline *tet*X4 and 7 *E. coli* with colistin resistance *mcr-1* gene. One *E. coli* PK9104 belonging to unknown ST carried both genes. However, long read confirmed that both genes are located on different plasmids. All the strains exhibited resistance to amoxicillin, tetracycline, tobramycin, ciprofloxacin, florfenicol, doxycycline, ampicillin and ciprofloxacin whereases carbapenem and cephalosporins were susceptible. The *mcr-1* was located on a IncI2 plasmid of ~6o kb size whereases tetX4 gene was located on IncFII MDR plasmid.

**Conclusions:** Our data showed high level of resistance to critically important antimicrobials in farm-to-fork integrated broiler production in Pakistan with both clonal and plasmid borne resistance mechanisms. The convergence of tigecycline and colistin resistance in *E. coli* in broiler production pyramid may pose a serious threat to human health.

**P-064**

# Brucella antimicrobial resistance is threating to close contact employees based on livestock vaccines and human isolates in northern China

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**Background and objectives:** Brucellosis, a chronic zoonotic disease caused by Brucella spp., poses significant global public health and economic challenges. Antibiotics are prescribed for treatment of human brucellosis. However, misuse of antibiotics and the inadequate treatment of infections have significantly increased the risk of resistance of Brucella to antibiotics, highlighting the urgent need for alternative therapeutic approaches.

**Methods:** Minimum inhibitory concentrations (MICs) were determined using the broth microdilution method, with susceptibility (S), intermediate (I), and resistant (R) categories defined according to CLSI standards. Livestock vaccines (A19, S2, BA0711, M5, RB51) and human brucella isolates (41 strains) originated from northern China were included in the study and 16 antibiotics were used to assess the MICs. Synergistic effects of three antibiotic combinations were assessed using the fractional inhibitory concentration index (FIC) .

**Results:** Old attenuated vaccines (A19, S2, and M5) yielded wide spectrum of antimicrobial resistance compare to new live strains (RB51 and BA0711). Obviously, S2 strains shown higher resistant to the first-line antibiotics, such as doxycycline (R≥2 μg/mL), streptomycin (MIC=32 μg/mL) and ceftriaxone (MIC=8 μg/mL). Regarding human isolates, 100%, 85.4%, 82.9%, 80.5%, 78.0%, and 78.0% were determined to resist to tobramycin, gentamicin, ciprofloxacin, ceftriaxone, sparfloxacin, and trimethoprim, respectively. Interestingly, the A19, M5, and S2 vaccine strains were also resistant to sparfloxacin, while S2 and M5 were resistant to trimethoprim and ciprofloxacin. Moreover, antimicrobial susceptibility analysis of the three combination regimens revealed that the doxycycline in combination with rifampicin combination exhibited marked synergistic effects (91.7%), indicating an optimal choice for Brucellosis treatment.

**Conclusions:** Based on above evidences, antimicrobial resistances are transferred from livestock to close contact employees and human antibiotics have been banned for livestock use due to simultaneous resistance risk. Highly sensitive antibiotics (e.g., doxycycline, rifampicin, ofloxacin) and combination of doxycycline and rifampicin are recommended for the therapy of human brucellosis.

**Figure**

Table 1 Summary of antibiotic susceptibility results for 5 Brucella vaccine strains

| Vaccine Strain | Susceptible (S) | Intermediate (I) | Resistant (R) |
|---|---|---|---|
| A19 | Rifampicin, Doxycycline, Cefoperazone, Minocycline, Gatifloxacin, Tobramycin, Gentamicin, Ciprofloxacin, Moxifloxacin, Clarithromycin | | Ofloxacin, Levofloxacin Sparfloxacin, Erythromycin Compound Sulfamethoxazole |
| BA0711 | Rifampicin, Doxycycline, Sparfloxacin, Minocycline, Gatifloxacin, Tobramycin, Gentamicin, Moxifloxacin, Ciprofloxacin | Cefoperazone (MIC=2 μg/mL) | Ofloxacin, Levofloxacin, Erythromycin, Compound Sulfamethoxazole |
| M5 | Rifampicin, Doxycycline, Cefoperazone, Minocycline, Tobramycin, Gentamicin (borderline sensitive) | Gatifloxacin (MIC=2 μg/mL) | Ofloxacin, Levofloxacin, Sparfloxacin, Tobramycin, Erythromycin, Moxifloxacin, Ciprofloxacin, Clarithromycin, Compound Sulfamethoxazole |
| RB51 | Doxycycline, Cefoperazone, Minocycline, Gatifloxacin, Tobramycin, Gentamicin, Ciprofloxacin | Ofloxacin, Levofloxacin, Sparfloxacin (MIC=2 μg/mL) | Rifampicin (highly resistant, MIC>128 μg/mL), Erythromycin, Moxifloxacin, Clarithromycin, Compound Sulfamethoxazole |
| S2 | Rifampicin, Minocycline, Tobramycin, Gentamicin | Gatifloxacin (MIC=2 μg/mL) | Doxycycline (MIC=2 μg/mL), Ofloxacin, Levofloxacin, Sparfloxacin, Cefoperazone, Gentamicin, Erythromycin, Moxifloxacin, Ciprofloxacin, Clarithromycin, Compound Sulfamethoxazole |

## P-065

# Identification of a chromosome-borne oxazolidinone resistance gene *poxtA* in *Lactobacillus salivarius* of chicken origin

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**Background and objectives:** The emergence of the phenicol-oxazolidinone-tetracycline resistance gene *poxtA* poses a significant challenge to public health[1]. Here, a *poxtA*-harbored *Lactobacillus salivarius* isolate of chicken origin was characterized.

**Methods:** The presence of mobile oxazolidinone resistance genes was determined by PCR assay[2]. Antimicrobial susceptibility testing was performed by broth microdilution and the results were interpreted according to EFSA guidelines[3]. Transfer experiments were carried out to assess horizontal transferability of the gene *poxtA*. WGS was performed using a combination of Oxford Nanopore MinION/Illumina HiSeq platforms.

**Results:** *Lactobacillus salivarius* isolate LS24 exhibiated multi-drug resistant phentype. PCR assay revealed that isolate LS24 was positive for *poxtA*. WGS showed that isolate LS24 contained a circular chromosomal DNA and seven plasmids. Sequence analysis indicated that *poxtA* gene was loacted in the chromosome and this observation was rarely reported until now. IS*Lasa1* element was inserted into the 3"-end of the *poxtA* gene, thereby disrupting the *poxtA* gene into two parts. Genetic environment analysis showed that except for a *fexB*-carrying fragment flanked by IS*1216E* in the same orientation, the *poxtA*-containing region was highly similar to the corresponding that of *L. salivarius* BNS11 of pig origin. Transfer experiment revealed that *poxtA* gene was not able to transfer via conjugation.

**Conclusions:** To the best of our knowledge, this is first report of chicken *Lactobacillus salivarius* harboring phenicol-oxazolidinone-tetracycline resistance gene *poxtA*. The occurrence of

mobile oxazolidinone resistance genes in *Lactobacillus* from food-producing animals needs close surveillance to prevent their spread to human pathogens.

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## P-066

# The role of efflux-mediated resistance in *Staphylococcus pseudintermedius,* a major animal pathogen

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**Background and objectives:** Background and objectives: *Staphylococcus pseudintermedius* is an opportunistic bacterium responsible for the majority of canine skin infections. The emergence of methicillin-resistant (MRSP) strains, linked to multidrug-resistance phenotypes, threatens the treatment options in veterinary settings. The role of efflux in antimicrobial resistance for this species remains largely overlooked. This study assessed the efflux-mediated response to ethidium bromide (EtBr) and tetraphenylphosphonium bromide (TPP), two known substrates of efflux pumps (EPs).

**Methods:** Methods: Three *S. pseudintermedius* clinical strains (two MRSP, one MSSP) and the type strain (DSM21284$^T$, MSSP) were submitted to stepwise adaptation to EtBr and TPP. The adapted strains were characterized regarding their susceptibility profile to eight antibiotics, five biocides and EtBr, both in the presence/absence of the efflux inhibitors (EIs) thioridazine and verapamil. EtBr accumulation assays were conducted in the presence/absence of glucose (energy source for EPs) and/or EIs. Both parental and adapted strains were further analyzed by whole-genome sequencing (WGS) analysis using Illumina MiSeq.

**Results:** Results: EtBr-adapted strains showed a decreased susceptibility to fluoroquinolones and biocides (TPP, benzalkonium chloride, chlorhexidine), whereas TPP-adapted strains revealed, in most cases, only reduced susceptibility to the same biocides. Reduced susceptibility to octenidine and triclosan was not detected. The EIs promoted a reduction (≥4-fold) in MICs of fluoroquinolones and biocides in the adapted strains, indicating increased efflux activity, later confirmed by EtBr accumulation assays. WGS analysis revealed changes in the promoter region of the *norA* gene encoding the main staphylococcal EP NorA.

**Conclusions:** Conclusions: This study demonstrated that in *S. pseudintermedius,* exposure to different antimicrobials can trigger an efflux-mediated response resulting in different phenotypes, including reduced susceptibility to biocides, such as TPP, benzalkonium chloride and chlorhexidine, the latter commonly used in the therapy of *S. pseudintermedius* infections.

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**P-067**

# Detection of oxazolidinone resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from a pig slaughterhouse, Italy

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**Background and objectives:** *Enterococcus* spp. frequently cause severe human infections that are difficult to treat due to widespread resistance to several antibiotics, including last-resort oxazolidinones. Although oxazolidinones are not approved for veterinary use, linezolid resistance genes are increasingly being detected in enterococci isolated from food-producing animals [1,2]. It"s well-known that this resistance may spread in livestock due to the use of phenicols [3]. Here, florfenicol-resistant *E. faecalis* and *E. faecium* isolates collected in a pig slaughterhouse were investigated for the occurrence of oxazolidinone resistance genes.

**Methods:** This study was conducted in 4 slaughter days, one for each season of the year, in a pig slaughterhouse in Central Italy. 33 cecal contents and 33 carcass swabs were collected each sampling day. Moreover, 22 environmental samples were collected before and after the slaughter. Only florfenicol-resistant *E. faecalis* and *E. faecium* were selected and analyzed by WGS to detect the presence of *cfr*, *cfr-like*, *optrA*, and *poxtA* genes. The isolates were subjected to MIC test and interpreted according to CLSI [4].

**Results:** 376 samples were collected, of which 97 (25.8%) tested positive for florfenicol-resistant *Enterococcus* spp. Environmental samples collected at the end of the day of the slaughter showed higher contamination levels compared to those collected beforehand ($p<0.05$). Genomic analysis of 44 *E. faecalis* and *E. faecium* isolates revealed a high prevalence of the *optrA* gene (79.6%), followed by *poxtA* (29.5%) and *cfr*(D) (11.3%). The *cfr*(D) gene was present only in *E. faecalis* isolates associated with *optrA* and/or *poxtA* genes (11.3%, 5/44). Twenty-four sequence types (STs) were identified, with ST59, ST21, and ST500 being predominant. The data indicated high resistance rates to tetracycline (93.2%) and erythromycin (72.7%), consistent with existing literature. Remarkably, linezolid resistance was observed in 72.7% of isolates, while no vancomycin resistance was detected.

**Conclusions:** Our study highlights the widespread presence of oxazolidinone resistance genes in *E. faecalis* and *E. faecium* isolates from a pig slaughterhouse in Central Italy, with *optrA* being the most prevalent. These findings underscore the need for continuous surveillance and prudent antimicrobial use in livestock to preserve the effectiveness of linezolid in the clinical setting.

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## P-068

# Novel integrative and conjugative elements carrying *cfr*(B) and *cfr*(C) linezolid resistance genes in *Clostridioides difficile* isolates from calves, Italy

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**Background and objectives:** The goal of the present study was: *(i)* to clarify the genetic basis responsible for the high linezolid MICs exhibited by three bovine *C. difficile* strains; *(ii)* to examine the involved genetic environments; and *(iii)* to compare their genomes with those available in databases to define the phylogenetic relationships among the isolates.

**Methods:** WGS was used to characterize the linezolid resistant *C. difficile* A501, A505, and A516 strains previously described in Blasi *et al.* [1]. Transferability of linezolid resistance was assessed by filter mating experiments.

**Results:** WGS analysis revealed the presence of *cfr*(B) in *C. difficile* A501 and A516, both included in the ST11. This ST is known to colonize and infect livestock worldwide, as well as cause disease in humans, highlighting its zoonotic potential [2]. In *C. difficile* A505, belonging to non-toxigenic ST15 clone, the *cfr*(C) was described [3]. The phylogenetic analysis showed that the A501 and A516 *C. difficile* strains clustered with other toxigenic strains belonging to the ST11, while A505 grouped with non-toxigenic ST8 and ST15. *cfr*(B) gene was on a novel 25,791-bp integrative conjugative element (ICE), named ICECd-*cfr*(B), similar to an uncharacterized region of *Clostridium* sp. C1, but significantly different from Tn*6218* transposon typically associated with this gene. The *cfr*(C) gene was found on a novel 32,770-bp ICE, named ICE*Cd*-*cfr*(C), which was identical to an uncharacterized region of the *C. difficile* DSM 104450 chromosome. ICE*Cd*-*cfr*(C) exhibited high nucleotide identity, but low coverage, with a *cfr*(C)-carrying region previously detected in *C. difficile* 020482; while in *C. difficile* A505 this region was interrupted by a 16.6-kb DNA insertion. Conjugation assays failed to demonstrate the transferability of *cfr*(B) and *cfr*(C) genes.

**Conclusions:** To the best of our knowledge, this is the first report of *C. difficile* isolates from calves with high linezolid MICs due to novel *cfr*(B)- and *cfr*(C)-carrying ICEs. *C. difficile* animal isolates, belonging to ST11 and ST15 clones with zoonotic potential, could act as reservoir for the spread of linezolid resistance genes to human intestinal pathogens, with serious consequences for public health.

**References**[1] Blasi F, Lovito C, Albini E et al. (2021) Animals (Basel); 11: 515.[2] Knight DR, Kullin B, Androga GO et al. (2019) mBio; 10: e00446-19[3] Okada Y, Yagihara Y, Wakabayashi Y et al. (2019) Access Microbiol; 2

**P-069**

## Regulation of Plasmid-Mediated Antibiotic Resistance and Gene Amplification by Chromosomal ampR in *Enterobacter cloacae*

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**Background and objectives:** Gene duplication and amplification (GDA) play a crucial role in bacterial adaptation to antibiotic pressure [1]. In *Enterobacter cloacae* strain IMT1, GDA is induced under ceftazidime selection, leading to the amplification of a genomic fragment containing *blaDHA* and its adjacent *ampR(DHA) [2]*. Additionally, the strain harbors plasmid-borne *blaTEM* and *blaCTX*, while the chromosome encodes *blaACT* alongside *ampR(ACT)*. Given the regulatory role of *ampR* in β-lactamase expression, we aimed to investigate whether *ampR*(ACT) influences GDA and the expression of plasmid-borne resistance genes, thereby modulating antibiotic susceptibility and bacterial fitness.

**Methods:** Gene deletion mutagenesis of *ampR(ACT)* was performed using λ Red mediated recombineering, followed by PCR and sequencing verification. Antibiotic susceptibility was determined using the agar disc diffusion assay. To assess GDA copy number, genomic DNA (gDNA) was extracted and analyzed using qPCR. Meanwhile, RNA was extracted and the expression levels of *blaDHA* and *ampR*(DHA) were quantified by RT-qPCR. Bacterial adaptation was further examined through ScanLag analysis of appearance time and growth time.

**Results:** Deletion of *ampR(ACT)* resulted in an unexpected increase in ceftazidime resistance despite a reduction in GDA copy number. RT-qPCR analysis showed upregulation of *blaDHA* and downregulation of *ampR(DHA)*, indicating altered regulation of β-lactamase expression. ScanLag analysis revealed prolonged appearance and growth times, suggesting a fitness trade-off associated with these regulatory changes.

**Conclusions:** These findings indicate that *ampR*(ACT) functions as a key regulatory element, influencing both chromosomal and plasmid-borne resistance genes. This study provides new insights into the genetic and regulatory mechanisms shaping antibiotic resistance evolution in *E. cloacae*.

**P-070**

## Epidemiology, Antimicrobial Resistance Profiles and Source of *Campylobacter* spp. Isolates from Pediatric Diarrheal Patients in Zhejiang Province, China

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**Background and objectives:** Campylobacter spp. is a leading cause of pediatric diarrhea, yet its epidemiology and antimicrobial resistance (AMR) mechanisms in children remain poorly understood in China. This study aims to investigate the epidemiology, antimicrobial resistance patterns, and potential sources of Campylobacter spp. infections in children from Zhejiang Province over a three-year period.

**Methods:** A total of 3,491 fecal samples from pediatric diarrheal patients were collected from two hospitals between 2017 and 2021. Campylobacter spp. isolates were identified using culture-based methods and MALDI-TOF-MS. Whole-genome sequencing was performed on Illumina NovaSeq PE150, followed by annotation, and multilocus. Antimicrobial susceptibility testing was conducted via the broth microdilution method. A comparative source attribution analysis was conducted using SNP analysis, incorporating publicly availableCampylobacter sequences from poultry, bovine, and swine sources to trace potential transmission pathways.

**Results:** In this three-year genomic epidemiology study (2017–2021), we analyzed Campylobacter isolates from diarrheal children in Zhejiang Province and identified Campylobacter jejuni as the dominant species, with an increasing prevalence from 2.80% in 2017 to 4.50% in 2021 (overall 3.49%). Whole-genome sequencing revealed a heterogeneous population structure, with ST21 CC (14.88%), ST353 CC (10.74%), and ST464 CC (10.74%) as dominant clonal complexes. Notably, we discovered two novel antimicrobial resistance genomic islands associated with multidrug resistance (MDR), conferring high-level resistance (>90%) to florquinolones and tetracyclines, two commonly used clinical treatments. Comparative phylogenomic analysis confirmed chicken as the primary infection source for pediatric campylobacteriosis, with RE-cmeABC acting as a key determinant of both MDR and cross-species transmission. Fixation index (Fst) analysis further demonstrated that RE-cmeABC gene flow is primarily mediated by homologous recombination, providing novel insights into host adaptation mechanisms.

**Conclusions:** These findings suggest that florquinolones and tetracyclines may no longer be suitable for treating pediatric campylobacteriosis and highlight the urgent need for revised treatment guidelines. Furthermore, the identification of RE-cmeABC as a genetic signature of host adaptation underscores the importance of controlling Campylobacter in poultry to mitigate MDR dissemination and zoonotic transmission. This study provides the first genomic-based evidence of RE-cmeABC-driven cross-species transmission in China, emphasizing the need for enhanced surveillance and targeted interventions to reduce pediatric Campylobacter infections.

**P-071**

## Evolutionary Adaptations and Resistance in *Escherichia coli* ST744

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**Background and objectives:** *Escherichia coli* sequence type (ST) 744 is a globally distributed, high-risk zoonotic lineage associated with extraintestinal infections and antimicrobial resistance. We aimed to investigate the genetic and phenotypic traits that may contribute to its successful spread and persistence across niches.

**Methods:** A phylogenetic analysis of 913 strains was performed, the genomes were screened for relevant genomic markers, and comparative genomics with MG1655 was conducted using two long-read sequenced strains. Thirty-two strains were then phenotypically characterised under optimal and stress conditions. Growth curves were used to evaluate adaptive responses influenced by ST744-

associated stress-coping and metabolism-related genes, with comparisons to MG1655 and other extra-intestinal pathogenic *E. coli* (ExPEC) strains.

**Results:** Two main clusters of ST744 linked to dominant serotypes (H9:O101, H10:O101) were identified. Despite the absence of typical ExPEC virulence genes, 170 clinically relevant strains (e.g., from blood or urine) were detected. In contrast, 96% of ST744 carried antibiotic resistance genes to at least three antibiotic classes, averaging 11 genes per strain. Genes for mercury and disinfectant resistance were also identified. Comparative genomics identified a distinct variable region in ST744, absent in MG1655, with *betU* gene possibly implicated in osmoprotection. Additionally, ST744 contained cold-shock proteins preventing RNA degradation at low temperatures; some are not found in several typical ExPEC lineages. Growth analysis indicated that *betU* does not affect fitness under osmotic stress, and limited fitness costs at suboptimal temperatures may relate to cold shock protein presence.

**Conclusions:** *E. coli* ST744 not only shows high antibiotic resistance but also displays atypical traits for ExPEC. Our findings suggest that this lineage has genetic adaptations that enhance its survival ability and endurance in various environments. Understanding its phenotypic characteristics and stress responses could provide essential insights into the factors driving ExPEC's emergence and success.

## P-072

# Dynamic changes in the plasmidome and resistome in the gastrointestinal tract of chickens

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**Background and objectives:** Intensive poultry farming has led to increased antibiotic use, driving the accumulation of antibiotic resistance genes (ARGs) in the chicken gut microbiome. This environment facilitates ARGs transfer via mobile genetic elements, potentially spreading resistance to other bacteria, including pathogens. This study aimed to analyze the composition and dynamics of plasmids and ARGs in the chicken gut.

**Methods:** Long-read sequencing was used to analyze total plasmid DNA from 12 fecal samples from three commercial chicken houses over the first four weeks post-hatching. Plasmid DNA was extracted using two isolation kits to capture both small and large plasmids, treated with DNase to remove linear fragments, and amplified with phi29. For comparison, metagenomic analysis of total DNA was

performed using short-read sequencing. All chickens received enrofloxacin early in life, with one house additionally treated with sulfamethoxazole/trimethoprim.

**Results:** A total of 24 antibiotic resistance gene classes were identified. Fluoroquinolone resistance genes were widespread across all houses, while diaminopyrimidine resistance genes were elevated in the treated house. The highest total RPKM value of plasmids among all samples in the metagenome analysis was 129.48, while the lowest in the plasmidome was 80 503.15. Complete plasmids carrying ARGs ranging from 2.6 to 47.6 kb in size were reconstructed. Most ARGs in the chicken gut microbiome were found on small plasmids, possibly due to the higher degradation of large plasmids during extraction, greater copy numbers of small plasmids, phi29 amplification bias, or microbiome composition. The most occurring plasmid across the samples was a 3 kb long MOBP-like plasmid of rep_cluster_2335 containing *qnrB46*-like|pan_9298 gene.

**Conclusions:** Resistance profiles reflected antibiotic treatments, reinforcing the role of the chicken gut as a reservoir of resistance genes. Plasmidome analysis allowed us to identify a wide range of variable plasmids and link them to ARGs. These findings highlight the necessity of targeted plasmid sequencing to improve the accuracy of horizontal gene transfer assessments.

## P-073

# Novel multidrug-resistance plasmid in methicillin-resistant *Mammaliicoccus lentus* from sheep in different farms

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**Background and objectives:** *Mammaliicoccus* species, which are members of *Staphylococcaceae*, are part of the natural skin microbiota of mammals. They are able to acquire virulence and antibiotic resistance genes making some of them difficult to treat opportunistic pathogens. Methicillin-resistant *Mammaliicoccus lentus* (MRML) isolates have recently been identified in Algerian sheep. In this study, a multidrug-resistance (MDR) plasmid identified in *M. lentus* from sheep in different farms, was characterized and analyzed for genetic relationship to previously identified plasmids using WGS-based comparative analysis.

**Methods:** Twelve *M. lentus* from different sheep and farms were sequenced using Illumina short-read and MinION long-read technology and hybrid assembled using Unicycler v0.5.0 to generate fully circular chromosome and plasmid sequences. Single nucleotide polymorphisms (SNPs) were called using Snippy version 4.6.0 (https://github.com/tseemann/snippy). Antimicrobial resistance genes and plasmids were *in silico* detected using ABRicate v1.0.1 (https://github.com/tseemann/abricate) against ResFinder and PlasmidFinder databases. Comparative analysis using BLASTn v2.9.0 for sequence similarity searches; and visualization was performed using Clinker v.0.0.28. For plasmids phylogeny, all available *M. lentus* plasmid sequences from the public plasmid database (PLSDB) were included. Sequences were aligned with MAFFT v7.453, and a maximum-likelihood tree was constructed using IQ-TREE v2.3.6 to determine the plasmids evolutionary placement.

**Results:** Four MRML strains from different sheep and farms (differing by two SNPs) were found to harbor a novel MDR plasmid (pCBS346) carrying six distinct antibiotic resistance genes. Plasmid pCBS346 carried tetracycline resistance genes (*tet*(L), *tet*(K)), trimethoprim (*dfrK*), macrolide–lincosamide–streptogramin B (*erm*(B)), and aminoglycosides (*ant(4')-Ia, str*) (Fig 1). BLASTn comparison revealed low similarity to known plasmids with ~48% sequence coverage with the closest plasmid of *M. lentus* Dog26 from a dog in Africa (GenBank accession no. CP120156) and ~46% with a plasmid from *Staphylococcus aureus* ST398 from cattle in Germany (GenBank accession no. FN806789) (Fig 1). This low homology highlights the unique genetic composition of the new plasmid. Phylogenetic analysis including all publicly available *M. lentus* plasmids revealed that the pCBS346 formed a distinct cluster with the plasmid of *M. lentus* Dog026. Comparative plasmid analysis also identified insertion sequence (IS) elements indicating several recombination events (Fig 1).

**Conclusions:** This study identified a novel MDR plasmid, pCBS346, in *M. lentus* from sheep, highlighting the role of livestock in the spread of antibiotic resistance. The presence of pCBS346 in clonally related strains from different farms suggests that it is rather spreading via clonal dissemination than by transfer. The unique genetic mosaic structure of plasmid pCBS346 suggests ongoing recombination potential.

## P-074

# A Decade of *Salmonella* Resistance in the French Pig and Pork Industry: Antibiotics, Heavy Metals, and Farm-to-Fork Transmission

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**Background and objectives:** The emergence of antibiotic-resistant *Salmonella* in the pig and pork industry poses a growing public health challenge, as this pathogen remains a major cause of foodborne infections associated with pork consumption in France and across the European Union (EU). Since the EU prohibited the use of antibiotics as growth promoters in 2006, heavy metals such as copper, zinc, and silver, among others, have been utilized for their antimicrobial properties and as growth promoters throughout the production chain. In Gram-negative bacteria, resistance genes for antibiotics and heavy metals have been reported to co-occur and, in some instances, co-localize on mobile genetic elements, potentially promoting their persistence and spread. However, the mechanisms underlying this co-selection in *Salmonella* remain poorly characterized. This study, conducted within a One Health framework, aims to investigate heavy metal and antibiotic resistance genes, their co-localization, and potential cross-resistance mechanisms.

**Methods:** A total of over 800 *Salmonella* strains, collected from the pig and pork production chain and human clinical cases between 2014 and 2024, were subjected to whole-genome sequencing using Illumina technology. Phylogenetic and pangenome analyses were performed to assess genetic diversity and the distribution of core and accessory genes. The resistome was characterized using multiple public databases, while the mobilome including plasmids, transposons, integrons, and integrative and conjugative elements was examined to predict possible co-localization of resistance determinants.

**Results:** The predominant *Salmonella* serovars identified across the pig and pork production chain in this studied panel included *S. Typhimurium*, its monophasic variant, and *S. Derby*, alongside 26 additional serovars. Serovar effective varied across production stages, with the highest distribution observed at the livestock and slaughterhouse levels. Preliminary analysis revealed that antimicrobial resistance genes against aminoglycosides, penicillin, tetracyclines, and sulfonamides were the most frequently detected in *Salmonella* isolates. Notably, genomic analyses revealed no resistance genes associated with carbapenemase.

**Conclusions:** Ongoing genomic analyses are focused on identifying antibiotic- and heavy metals-resistant strains and assessing their potential for horizontal gene transfer, as well as possible cross-resistance between antibiotics and heavy metals commonly used in pig farming. These aspects will be further explored through long-read sequencing and phenotypic assays. The findings of this study will contribute to a better understanding of the persistence and spread of resistant *Salmonella* within pig and pork production systems in France and may inform targeted strategies to mitigate antimicrobial resistance.

## P-075

# Network and core genome analyses of 3GC-R IncI1-I(alpha) plasmids from a multi-species international collection reveals geographic- and/or species-specific plasmid evolution

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**Background and objectives:** Incompatibility group I1 (IncI1) plasmids have been globally isolated from different Enterobacterales from a wide range of animal and human origin. They have gained attention due to their widespread carriage of third-generation cephalosporin resistance (3GC-R) encoding genes posing a public health threat. To place plasmids into a geographical and host context, a network analysis and a core plasmid genome phylogeny was reconstructed and their species and geographical region association assessed.

**Methods:** A total of 691 3GC-R IncI1-I(alpha) plasmids available in the PLSDB and the GenBank were used for this analysis. Their recombination-aware similarity was assessed by performing a network analysis using pling v.2.0.0. Plasmids (n=691) were reannotated using Bakta v.1.9.4 and a plasmid pangenome analysis was performed using panaroo v.1.5.1 (core threshold 95%). A core alignment (20 genes) resulting from the pan-IncI1-I(alpha)-plasmids-genome analysis was used to reconstruct a global phylogeny using FastTree v.2.1.11 and its correlation with the most prevalent network subcommunities resulting from the network analysis was assessed. ARGs were screened *in silico* using ABRicate v.1.0.1.

**Results:** The major network subcommunities of 3GC-R IncI1-I(alpha) plasmids identified based on a containment distance of 0.3 and threshold of two recombination events (2 DCJ-Indel) correlated with the backbone-based phylogeny. A first subcommunity (SC) (SC 441) consisted of 71 plasmids belonging to a phylogenetic lineage harboring *bla*CMY-2 which has been mostly isolated from *Salmonella* and *Escherichia* species in North America and Asia. Two subcommunities, SC 440 (n=47) and SC 438 (n=37), belonged to a plasmid lineage carrying almost exclusively *bla*CTX-M-1 which has been found worldwide mostly among *Escherichia coli*. Another major subcommunity (SC 439, n=41) belonged to a lineage detected almost exclusively among *E. coli*, which carried mostly *bla*CMY-42, and has spread among different continents. Another subcommunity (SC 437, n=25) was part of a lineage harboring *bla*CTX-M-55 (a sub-lineage/subcommunity 430 acquired instead of *bla*CTX-M-15) that has been detected mostly in Asia among different species including *E. coli*, *S. enterica*, *Shigella sonnei*, *Klebsiella pneumoniae* and *Enterobacter hormaechei*. Of note, several sub-lineages formed minor subcommunities indicating further evolution driven by mutations and recombinations. Most lineages carried several additional ARGs.

**Conclusions:** This study shows the evolutionary trajectory of 3GC-R IncI1-I(alpha) plasmids highlighting certain lineages/subcommunities associated with specific 3GC-R genes, bacterial species and/or geographical regions and demonstrates the need to use phylogenetic and recombination-aware methods to fully understand their evolutionary dynamics.

**P-076**

# Potential for efflux-mediated resistance in *Staphylococcus coagulans*

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**Background and objectives:** *Staphylococcus coagulans* is a common cause of skin and soft tissue infections in companion animals, often requiring the prescription of antimicrobial agents. While efflux is already established as the first-line response to antimicrobial agents and biocides in other pathogenic staphylococci, data on efflux-driven resistance in *S. coagulans* is still scarce. This work aims to explore the potential of efflux to mediate antimicrobial and biocide resistance in *S. coagulans*.

**Methods:** The strain *S. coagulans* DSM6628$^T$ was adapted to ethidium bromide (EtBr, a substrate of a wide range of multidrug resistance efflux pumps in staphylococci) by a step-wise process, exposing the strain to doubling EtBr concentrations (0.125-32 mg/L). The parental and the EtBr-adapted strain, DSM6628_EtBr, were evaluated for their antimicrobial and biocide susceptibility by minimal inhibitory concentration (MIC) determination. Efflux activity was evaluated by (i) re-determination of antimicrobial and biocide MICs in the presence of known efflux inhibitors, verapamil (VER) and thioridazine (TZ), at ¼ of their MIC; (ii) EtBr accumulation real-time fluorometric assays in the presence and absence of glucose, VER and TZ.

**Results:** Strain *S. coagulans* DSM6628_EtBr was obtained after nine passages in EtBr and exhibited a MIC of 32 mg/L, a 128-fold MIC increase relative to the parental strain. It also showed reduced susceptibility (2-8 fold MIC increases) towards the hydrophilic fluoroquinolone norfloxacin and several biocides, including chlorhexidine, the quaternary ammonium compounds benzalkonium chloride, dequalinium chloride and cetylpyridinium chloride, as well as to tetraphenylphosphonium bromide. The efflux inhibitors restored the norfloxacin and biocide MICs to the levels shown by the parental strain. The fluorometry assays confirmed the increased efflux activity of the EtBr-adapted strain, especially in the presence of glucose, an energy source for efflux pumps. Ongoing work focuses on gene expression assays and genomic analysis to identify the efflux pump(s) involved and genomic alterations contributing to this efflux-mediated phenotype.

**Conclusions:** This work demonstrates the role of efflux in reduced susceptibility phenotypes to antimicrobial agents and biocides in *S. coagulans*, highlighting the need for further research of this resistance mechanism in this relevant animal pathogen.

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**P-077**

# Identification of two novel phenicol resistance plasmids in bovine *Pasteurella multocida* from Germany

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**Background and objectives:** *Pasteurella multocida* can cause various infections in different host species, including humans. In cattle, this pathogen is frequently involved in the multifactorial bovine respiratory disease (BRD). Although the use of chloramphenicol in food-producing animals was banned in the European Union in 1994, its fluorinated derivative florfenicol was approved in 1995 for the treatment of BRD. Antimicrobial resistance (AMR) in *P. multocida* is of growing concern and plasmids have been identified to be involved in the dissemination of AMR genes in this organism. In this study, we characterized two novel phenicol resistance plasmids from *P. multocida*.

**Methods:** Overall, 179 bovine *P. multocida* from the German national resistance monitoring GE*RM*-Vet 2010-2022 were subjected to whole-genome sequencing on Oxford Nanopore MinION and Illumina MiSeq platforms, followed by hybrid assembly and sequence analysis. Plasmid DNA was additionally extracted via alkaline lysis and visualized by gel electrophoresis. Antimicrobial susceptibility testing (broth microdilution, agar disk diffusion) was performed according to CLSI recommendations.

**Results:** *P. multocida* 170411 and 190067 each carried a novel phenicol resistance plasmid, designated pHKH170411 and pHKH190067, respectively. Plasmid pHKH170411 (15,822 bp) was larger in size than plasmid pHKH190067 (5,727 bp), but both carried the *floR* gene, coding for a phenicol exporter. The novel plasmids shared similarities with previously described plasmids from *Pasteurellaceae* associated with BRD: pCCK381 (10,874 bp) and p103220 (11,053 bp) from *P. multocida* as well as pCCK13698 (14,969 bp, multiresistance plasmid) from *Bibersteinia trehalosi*. Although no additional AMR genes were identified on the novel plasmids, both isolates harbored another chromosomal resistance region. Here, *P. multocida* 170411 harbored a second *floR* copy and the AMR genes *sul2* (sulfonamides), *erm*(T) (macrolides-lincosamides-streptogramin B) and *tet*(Y) (tetracycline), whereas *P. multocida* 190067 carried *tet*(H) (tetracycline), *aadA31* (spectinomycin/streptomycin), *sul2*, *strA* (streptomycin), *strB* (streptomycin) and *aphA1* (kanamycin/neomycin). The phenotypic antimicrobial susceptibility testing results corresponded to the observed AMR genotype.

**Conclusions:** In summary, two novel *floR*-carrying phenicol resistance plasmids have been identified in bovine *P. multocida* isolates from Germany. They are composed of several segments previously found on other plasmids in *Pasteurellaceae* associated with BRD, highlighting the importance of interplasmid recombination processes possibly facilitating the spread of AMR determinants. Although recent data from GE*RM*-Vet indicate a comparatively low and stable florfenicol resistance rate of < 5% for *P. multocida* from cattle in Germany, our findings highlight the risk of limited therapeutic options in the control of BRD due to the further dissemination of phenicol resistance via horizontal gene transfer of mobile genetic elements, such as plasmids.

**P-078**

# Identification of the novel multiresistance transposon Tn*7731* in bovine *Pasteurella multocida* from Germany

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**Background and objectives:** *Pasteurella multocida* is one of the bacterial pathogens involved in the multifactorial bovine respiratory disease (BRD), but can also cause infections in various other host species including humans. Numerous mobile genetic elements have been identified to be involved in the dissemination of antimicrobial resistance (AMR) genes in *P*. *multocida*. Among the transposons, only the composite transposon Tn*5706*, native to *P*. *multocida*, has been identified so far. Here, we characterized a novel multiresistance transposon from *P*. *multocida*.

**Methods:** In total, 179 bovine *P*. *multocida* from Germany obtained in the national resistance monitoring GE*RM*-Vet 2010-2022 were subjected to whole-genome sequencing on Illumina MiSeq and Oxford Nanopore MinION platforms. Hybrid assembly followed by sequence analysis revealed that isolate 200011 from 2020 carried two copies of a novel transposon, designated Tn*7731* by the Transposon Registry. Antimicrobial susceptibility testing via broth microdilution, broth macrodilution and/or agar disk diffusion was performed according to CLSI recommendations.

**Results:** *P*. *multocida* 200011 carried two identical copies of the novel Tn*10*-related transposon Tn*7731* in opposite orientations. Tn*7731* was 9,701 bp in size and harbored the IS*10* insertion sequences IS*10L* and IS*10R* in opposite orientations at its termini. Moreover, Tn*7731* carried the four complete AMR genes *sul2* (sulfonamides), *catA3* (chloramphenicol), *strA* (streptomycin) and *tet*(B) (tetracycline), as well as a truncated *strB* gene (streptomycin). A 13,216-bp transposon-like element from a BRD-associated *Gallibacterium anatis* strain closely resembled Tn*7731* in the initial 3,172 bp and in the final part 3,837–9,701 bp. Between these sections, *catA3* in Tn*7731* replaced the AMR gene cluster *floR*-IS*15DII-aadB-aphA1* present in *G*. *anatis*. Two possible recombination sites of 45 bp upstream and of 7 bp downstream of *catA3* have been identified that might have served for the replacement of the cluster by *catA3* in a transposon-like element similar to that from *G*. *anatis*, thereby resulting in the novel Tn*7731*. Notably, isolate 200011 carried another chromosomal AMR region including the genes *sul2*, *catA3*, *strA*, *strB*, *floR* (chloramphenicol, florfenicol), *erm*(T) (macrolide-lincosamide-streptogramin B), and *tet*(Y) (tetracycline). Antimicrobial susceptibility testing results corresponded to the observed AMR genotype, with the exception of comparatively low tetracycline minimal inhibitory concentrations. However, explanatory mutational changes were not detected in the promoter regions of the *tet*(Y), *tet*(B) or associated *tetR* genes.

**Conclusions:** A novel composite multiresistance transposon of the Tn*10* family, designated Tn*7731*, was found in a bovine *P*. *multocida* isolate from Germany. This finding is important, because chromosomally located multiresistance transposons in bovine respiratory tract pathogens might limit therapeutic options in the control of BRD, one of the economically most relevant diseases in cattle.

**P-079**

# Long-term environmental persistence and evidence for multi-species horizontal transmission of an IncN ST7 plasmid carrying the *bla*$_{VIM-1}$ metallo-β-lactamase gene on a broiler farm in Austria

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**Background and objectives:** Carbapenemase-producing *Enterobacterales* (CPE) pose a major threat to public health and recent data indicate that CPE are spreading among EU livestock populations. Here, trace-back investigations on a broiler farm and molecular characterization of plasmids carrying the *bla*$_{VIM-1}$ metallo-β-lactamase gene are shown.

**Methods:** Specific isolation of ESBL/AmpC/carbapenemase-producing *Escherichia coli* was done according to the protocol defined by the EU reference laboratory (EURL) for antimicrobial resistance. For screening of *Enterobacterales* additional incubation at 37°C and a PCR assay were used. Short-read and long-read sequencing of isolates was done on Illumina MiSeq and Oxford Nanopore GridION sequencing platforms followed by assembly and sequence analysis. Antimicrobial susceptibility testing via broth microdilution was performed according to CLSI standards.

**Results:** Within the harmonized European monitoring for AMR (CID 2013/652/EU and (EU) 2020/1729)), a VIM-1-producing *E. coli* ST1196 was isolated from broiler caeca in Austria, in 2020. Broilers from the same producer were found positive for *E. coli* (ST154, ST155, ST206, ST679, ST1196) carrying *bla*$_{VIM-1}$ in subsequent samplings over a period of four years. In 2023, trace-back investigations on the farm showed that chicks that previously tested negative became positive for VIM-1-producing *E. coli* ST155 and *Klebsiella pneumoniae* ST873, respectively, within a few days after placement. Environmental sampling (floor, walls, drinkers, feeders, air inlet) identified various species and sequence types harbouring the gene *bla*$_{VIM-1}$ after clean-up (*E. coli, K. pneumoniae*) and disinfection (*Citrobacter* sp., *Atlantibacter subterranea*, and *Enterobacter hormaechei*) of the broiler house, respectively. Isolates showed reduced susceptibility to carbapenems. Hybrid assemblies of 11 selected isolates from broilers and the environment demonstrated that the gene encoding VIM-1 was located on an IncN ST7 plasmid with multiple antimicrobial resistance genes. Using the plasmid from *E. coli* ST1196 (2020) as reference, all except one IncN ST7 plasmids of different strains or species were very similar with 99.99 to 100% sequence identity and 99 to 100% coverage.

**Conclusions:** For the first time, *Enterobacterales* carrying a plasmid-borne *bla*$_{VIM-1}$ metallo-β-lactamase gene were detected on a broiler farm in Austria. The plasmid´s persistence over a period of four years and its presence in different species indicate high stability and evidence for multi-species transfer of the plasmid which seems to pose a serious problem for effective control of CPE.

**P-080**

## Comparison of *Escherichia coli* conjugation rates under aerobic and anaerobic environments

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**Background and objectives:** Plasmids play an important role in the dissemination of antibiotic resistance, a growing public health concern. Various factors are known to influence the conjugation rate of plasmids, including temperature, pH and cell density. Yet little is known about how anaerobic conditions affect plasmid conjugation rates in facultative anaerobic bacteria such as *Escherichia coli*. Even though many of their natural environments, such as the intestinal tract, abscesses and biofilms, maintain predominantly anaerobic conditions. Therefore, to understand and mitigate the spread of antibiotic resistance in real-world settings, it is essential that we determine the effect of anaerobic environments on plasmid conjugation rates.

**Methods:** Three donor *E.coli* strains carrying an IncI1α plasmid with the $bla_{CTX\text{-}M\text{-}1}$ gene conferring resistance to cefotaxime, were paired with three recipient strains that had chromosomal mutations causing resistance to ciprofloxacin. This pairing resulted in a full factorial design with nine combinations. All strains were isolated from the chicken cecum. Conjugation assays were performed in liquid culture without antibiotics, by mixing exponential-phase cultures in a 1:1 ratio. The cultures were incubated for 5 hours and 3 hours for aerobic and anaerobic conditions, respectively, to maintain comparable growth phases. Putative transconjugants were selected on double selective media containing cefotaxime and ciprofloxacin. A subset of colonies were subsequently confirmed by $bla_{CTX\text{-}M\text{-}1}$ PCR. All experiments were conducted in triplicate and conjugation rates estimated with the Approximate Extended Simonsen Model (ASM) using R.

**Results:** Preliminary results indicate a difference in the rate of conjugation under aerobic versus anaerobic environments. For one recipient, the conjugation rate improved under anaerobic conditions with all donors, while for another recipient the conjugation rate decreased under anaerobic conditions.

**Conclusions:** These results suggest a diverse effect of anaerobic conditions on conjugation rates and highlight the important role that recipients play in bacterial conjugation.

**P-081**

## Formation of a novel multiresistance plasmid co-carrying tigecycline, carbapenem and other resistance genes by recombination during conjugative transfer in *Klebsiella pneumoniae*

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**Background and objectives:** *Klebsiella pneumoniae* is a common nosocomial pathogen, and the emergence of carbapenem-resistant *K. pneumoniae* (CRKP) has significantly limited therapeutic options, posing a serious threat to public health [1]. Tigecycline, a glycylcycline antibiotic, was introduced as a broad-spectrum agent effective against a wide range of multidrug-resistant pathogens, including CRKP. However, the emergence of tigecycline resistance has further exacerbated the already problematic situation, rendering this last-line treatment increasingly ineffective [2]. This study investigates the formation of a novel multiresistance plasmid co-carrying tigecycline, carbapenem, and other resistance genes in *K. pneumoniae* through recombination during conjugative transfer.

**Methods:** A multidrug-resistant *K. pneumoniae* strain co-harboring a *tet*(A) variant, *tmexCD2-toprJ2*, and *bla*NDM-1 was isolated from clinical samples. Whole-genome sequencing, conjugative transfer experiments, and PCR analysis were performed to investigate the genetic environment and transferability of the resistance genes. The formation of a recombinant plasmid was confirmed through sequence analysis and S1-PFGE.

**Results:** The *K. pneumonia* 20K-383 was found to carry the *tet*(A) variant, *tmexCD2-toprJ2*, and *bla*NDM-1 resistance genes on two plasmids, p20K383-1 and p20K383-2. Conjugation experiments revealed the transferability of these resistance genes, with a novel recombinant plasmid pTC383-1 formed in transconjugant TC383-1. This recombinant plasmid was created through the recombination of the *tet*(A) variant and *bla*NDM-1 genes, mediated by IS*26* and IS*3000* sequences, and integrated into the recipient plasmid pYZ6_P1 via homologous recombination.

**Conclusions:** This study highlights the crucial role of transposases in the formation of complex multidrug-resistant bacterial strains. The identification of the recombinant plasmid provides new insights into the mechanisms of resistance gene dissemination in *K. pneumoniae*, emphasizing the need for enhanced surveillance and control measures to prevent the spread of such resistance determinants.

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## P-082

# Genetic characteristics of linezolid resistance in MRSA from food and animals

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**Background and objectives:** Linezolid (LZD) is a last resort antibiotic applied for human treatment of severe Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus* (VRE) infections as well as for tuberculosis. Although not licensed for the treatment of food-producing animals, LZD-resistant MRSA have been reported in European livestock [1,2]. In this study, we characterised LZD-resistant MRSA from German livestock and food in order to understand their genetic diversity and possible transmission routes of this important antimicrobial resistance.

**Methods:** Since 2012, MRSA isolates from various food and animals have been provided by the federal state laboratories in Germany and have been characterised at the BfR regarding their phenotypic resistance using broth microdilution. Strains with a phenotypic LZD-resistance were further analysed by whole genome sequencing using Illumina short read sequencing and long read sequencing by Oxford Nanopore Technologies to create hybrid assemblies. Bioinformatic inhouse pipelines were used for antimicrobial genotyping, MLST, virulence gene detection and the detection and characterisation of plasmids.

**Results:** In total, five MRSA strains were phenotypically LZD-resistant. Three ST398 strains of *spa*-types t011, t034 and t899 originated from pork and a pig carcass, respectively, whereas two ST9-t899

MRSA were isolated from turkey meat and a turkey carcass. In addition to LZD, the MRSA isolates were phenotypically resistant against up to ten antimicrobial classes and were attributed to SCC*mec* types IVa and V. The most prevalent antimicrobial resistance genes that affect phenotypic resistance were *blaZ*, *cfr*, *fexA*, and *mecA*. Moreover, other resistance genes like *dfrG*, *dfrS1*, *fosB*, *lnu*(B), *lsa*(B), *lsa*(E), *str*, *tet*(K), *tet*(M), *vga*(A) and point mutations in the genes *glpT, gyrA, murA, parC* occurred. Furthermore, the t899-MRSA harboured metal resistance genes *mco* or *arsB*, and one of them additionally the biocide resistance gene *qacG*. Plasmids could be found in all MRSA strains and showed co-localisation of antimicrobial resistance genes like *cfr* and *fexA*. Further characterisation of the plasmids is in progress. The two ST9-t899 strains carried genes for important virulence factors like vWBP relevant for abscess formation and the *egc* with relevance for food poisoning whereas the ST398-t899 strain carried the immune evasion cluster genes *chp*, *scn,* and *sak*.

**Conclusions:** Plasmid-encoded and transferable LZD-resistance could be identified in five livestock-associated MRSA isolates with porcine and poultry origin and was associated with multi-resistance against up to eleven antimicrobial classes. Co-localisation of different antimicrobial resistance genes on plasmids occurred and indicated co-selection events during transmission. Further relevant virulence factors could be identified and point towards a relevance for human infectious diseases or food poisoning.

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## P-083

# Co-existence of *tet*(X4) and *mcr-1* in a hybrid Shiga toxin-producing and enterotoxigenic *Escherichia coli* strain

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**Background and objectives:** The emergence of the plasmid-mediated tigecycline resistance gene *tet*(X4) and the plasmid-mediated colistin resistance gene *mcr-1* poses a significant challenge to public health[1-2]. Here, a hybrid Shiga toxin-producing and enterotoxigenic *Escherichia coli* isolate co-harboring *tet*(X4) and *mcr-1* genes was characterized.

**Methods:** The presence of mobile resistance genes was determined by PCR assays. Antimicrobial susceptibility testing was performed by broth microdilution and the results were interpreted according to CLSI guidelines and EUCAST. Transfer experiments were carried out to assess horizontal transferability of plasmids. WGS was performed using a combination of Oxford Nanopore PromethION/Illumina NovaSeq platforms.

**Results:** *Escherichia coli* isolate MD31 co-carrying *tet*(X4) and *mcr-1* was isolated from piglets with diarrhea in China. PCR assays revealed that *E. coli* isolate MD31 was a STEC/ETEC hybrid strain. This isolate was resistant to almost all antimicrobials except meropenem. Both *tet*(X4) and *mcr-1* could be successfully conjugated into the recipient *E. coli* C600. WGS showed that isolate MD31 consisted of a circular chromosomal DNA and five plasmids. Sequence analysis indicated that *tet*(X4) was located on the IncFIA(HI1)/HI1A/HI1B hybrid plasmid pMD31-tetX4, and *mcr-1* was located on the IncI2 plasmid pMD31-mcr-1. The two plasmids exhibited high similarity to multiple plasmids belonging to the same incompatibility type from *E. coli*.

**Conclusions:** To the best of our knowledge, this is first report of a STEC/ETEC hybrid isolate co-harboring *tet*(X4) and *mcr-1*. More attention should be paid to the prevalence of pathogens carrying *tet*(X4) and *mcr-1*.

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## P-084

# Strengthening surveillance of antimicrobial-resistant pathogens in bivalve molluscs

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**Background and objectives:** As part of the monitoring of microbial resistance (AMR) in Senegal, the Fisheries Processing Industries Directorate, in collaboration with the FAO and the Institut Pasteur in Dakar, has carried out characterisation and sample collection missions on oysters and water at a number of the country's oyster-farming sites. Microbiological analyses were carried out at the Microbiology Unit of the Paster Institute in Dakar. The aim of this activity is to assess the sanitary level of oyster production areas and the sanitary quality of oysters at national level.

**Methods:** Enterobacteriaceae (Salmonella spp and E. coli) were detected after enrichment in Buffered Peptone Water (BPW) broth and inoculation on specific media. 25g of oyster flesh was diluted and ground, then mixed with 10mL of EPT and incubated at 37°C. After 18-24 hours of incubation, 1 to 2 drops were placed on BCP and/or Drigalski media and spread for isolation of E. coli. The search for clones resistant to B-lactamines (E. coli-BLSE) was carried out on Mac Conkey medium, supplemented with antibiotic (cefotaxime). At the same time, 1mL of EPT was mixed with 9mL of MKTTn broth and incubated again at 37°C overnight before inoculation on SS, Heacktoen and BCP media for Salmonella spp. Suspect E. coli and Salmonella spp colonies were subcultured to obtain pure cultures before final identification using MALDI-ToF (Bruker, Germany) and possibly API-20E biochemical test strips (BioMerieux, France).

Vibrio spp were detected after enrichment of 25g of mollusc flesh, previously dilacrated and ground, in Alkaline Peptone Water (EPA) broth. After an overnight incubation at 37°C, 1 to 2 drops were spread on TCBS agar and chromogenic agar (CHROMagar) for isolation of Vibrio spp. Suspect colonies were subcultured again to obtain a pure culture before final identification with the MALDI-ToF spectrometer (Bruker, Germany).

Antibiotic susceptibility testing was carried out on isolates of E. coli, Salmonella spp and Vibrio parahaemolyticus using the Muller Hinton agar antibiotic diffusion method based on the Kirby-Bauer technique and interpreted in accordance with the latest recommendations of the CA-SFM v.1. 2022.

**Results:** A total of 112 bacterial germs were identified in shellfish samples (n=50) and water samples (n=62), mainly in the central (Fatick-Thiès) and southern (Ziguinchor) zones, with 66 and 42 bacterial isolates respectively. Analysis of the mollusc samples showed significant contamination by E. coli and V. paraheamolyticus in products from Fatick and Ziguinchor. Other germs of medical interest were also found in samples from Fatick.

Antibiograms were performed only on isolates of E. coli, Salmonella spp and V. parahemolyticus and the results obtained show a predominance of wild phenotypes in E. coli and Salmonella spp, and sensitivity to practically all the antibiotics tested for V. parahaemolyticus. However, 4 clones of E. coli with a low level penicillinase were found in Fatick (n=3) and Ziguinchor (n=1), including one on oysters from the Soukouta site (Fatick). In addition, two clones of E. coli producing extended-spectrum B-

lactamase (E. coli-BLSE) were also found in oyster production at Niodior (Fatick) and in the waters of Tobor (Ziguinchor).

**Conclusions:** Microbiological analyses of water and shellfish samples collected as part of this AMR monitoring activity show a diversity of micro-organisms, dominated by Escherichia coli and Vibrio parahaemolyticus. Although the majority of these organisms are still sensitive to antibiotics, clones of E. coli resistant to B-lactamines have been isolated both in the environment and from oysters. Hence the need to step up AMR surveillance using an inclusive approach involving all players in the various sectors.

## P-085

# β-lactamase resistance plasmids convey no measurable fitness benefit in commensal *E. coli* broiler chicken isolates, but plasmid loss is often associated with toxicity

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**Background and objectives:** Resistance to commonly used β-lactam and cephalosporin antibiotics through plasmid-based carriage of extended-spectrum/broad-spectrum β-lactamase genes (ESBL/BSBL) has spread among bacteria in animal environments. When no antibiotics are present, plasmid carriage is thought to impose a fitness cost until host adaptation occurs. However, β-lactamase carriage is observed even in absence of selective pressure, albeit at a lower frequency. The backbone of resistance plasmids carries a plethora of genes, many of which have no known function. Previous studies have found both positive and negative impacts on bacterial fitness (Giles et al., 2018; Schaufler et al., 2016) from carriage of native β-lactamase plasmids. We thus investigated whether the continued carriage of antibiotics resistance plasmids in the absence of antibiotics pressure is enhanced by fitness benefits derived from the plasmid backbone. We test this hypothesis using a plasmid-borne CRISPR-Cas9 system for curing β-lactamase plasmids (pCBL) to remove ("cure") entire native ESBL/AmpC plasmids from commensal *E. coli* isolates derived from broiler chickens and their environment.

**Methods:** We constructed pCBL using standard microbiological techniques. B-lactam resistant *E. coli* strains from the broiler environment were electrotransformed with pCBL and cured via incubation in rhamnose-containing M9 medium. Growth characteristics during and after plasmid curing were assayed in microtiter plates. Biofilm formation was assessed using crystal violet adhesion assays and microcolony assays.

**Results:** We cured a range of *E. coli* strains carrying *bla*SHV, *bla*CMY, *bla*TEM, and *bla*CTX-M genes using pCBL. Successful curing was confirmed via a loss of resistance and via qPCR. In most strains, we see no fitness benefit in the wild type compared to the cured strains in liquid growth or biofilm formation. Instead, many wild type strains show a reduction in liquid growth, indicating carrying resistance plasmids has a fitness cost. However, we also find that plasmid curing is frequently too toxic for successful curing before mutational escape via CRISPR-cas9 inactivation occurs. This effect is less frequent in *bla*SHV strains and more frequent in *bla*CTX-M strains.

**Conclusions:** We were unable to confirm a consistent fitness benefit derived from ESBL/AmpC plasmid carriage in adapted commensal *E. coli* isolates from the broiler chicken environment. Our data indicate that persistence of β-lactam resistance in the absence of selective pressure may be related to the toxicity of plasmid loss, likely due to toxin-antitoxin systems, rather than due to fitness benefits of plasmid carriage.

**P-087**

# Forgotten IncZ plasmids – classification and occurrence

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**Background and objectives:** 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. IncZ plasmids are part of the I-complex together with IncB/O, IncI1, IncI2, IncI1ɣ, IncK1 and IncK2. Currently, IncZ plasmids cannot be detected or distinguished from other members of the I-complex, by the most commonly used tools like PCR-Based Replicon Typing (PBRT) and PlasmidFinder. Therefore, their occurrence and epidemiology are not well-known.

**Methods:** This study analysed 56 IncZ plasmids isolated from *E. coli* from humans, cattle, poultry and pigs in Europe. The isolates were mapped against reference IncZ plasmids using Blastn to detect the presence of the IncZ plasmid. The isolates were sequenced using both Illumina and Nanopore technology. Additionally, 126 publicly available complete sequences were added to the analysis.

**Results:** A phylogenetic tree based on the RNAI-sequence was constructed, revealing distinct clusters. The largest cluster contained IncZ plasmids isolated mostly from humans (n=67), but also animals, which indicates plasmids in this cluster are spread more widely. These plasmids harboured a large diversity of resistance genes. The second largest cluster contained IncZ plasmids (n=42), which were isolated exclusively from hospitalized patients and were associated with *bla*CTX-M-14. Other clusters detected were significantly smaller possibly indicating lower prevalence of plasmids within them, compared those in the afore mentioned two large clusters, or a sampling bias. Phylogenetic trees based on alignments of the PBRT amplicon and whole plasmid sequences were also constructed in order to compare plasmid clustering and to assess the possible application of each sequence as a typing target. Different clusters were observed between the different phylogenetic trees due to large number of accessory genes.

**Conclusions:** Data presented showed occurrence of IncZ plasmids, highlighting the importance of plasmid classification in its role for detection of prevalent and/or endemic plasmids. Additionally, the results obtained demonstrate difficulties with choosing appropriate targets for IncZ plasmid classification.

**P-088**

# Identification of a novel family of Enterobacteria phage spreading antimicrobial resistance genes among *Enterobacterales*

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**Background and objectives:** In the order *Enterobacterales*, acquired antimicrobial resistance genes spread mainly on conjugative elements such as plasmids and integrative elements. Phage genomes rarely encode antimicrobial resistance genes. Here, a novel temperate Enterobacteria phage carrying several antimicrobial resistance genes, notably *bla*CTX-M-55, was identified in *Escherichia coli* isolates from poultry. Genetic and phenotypic characterization of this novel phage was carried out.

**Methods:** Horizontal transfers were initially performed from *E. coli* field isolates into sodium-azid resistant recipient *E. coli* strain J5-3. Then, phage particles were purified by PEG/NaCl precipitation. Transduction assays were realized into different *E. coli* lab strains and *Enterobacterales* species to determine host range. Field strains, lysogenic recipients and phage lysates were analyzed phenotypically and genetically by antimicrobial susceptibility testing, transmission electron microscopy, plaque formation assays, and whole-genome sequencing.

**Results:** Two nearly-identical representatives of a novel Enterobacteria phage, named respectively phEC_B25FEP & phEC_M24FEP, were identified by horizontal transfer assays of the ESBL gene *bla*CTX-M-55 from 2 distinct *E. coli* donor strains of broilers. These phages were first identified as 72-kb prophages specifically integrated at the 3"-end of tRNA-Phe into the chromosome of lysogenic recipients. They harbored resistance to chloramphenicol/florfenicol (*floR*), tetracyclines (*tetA*), streptomycin (*aph(3"")-Ib*/*aph(6)-Id*, sulphonamides (*sul2*) and expanded-spectrum cephalosporins (IS*Ecp1*-*bla*CTX-M-55). Beside phage-specific genes, phages phEC_B25FEP & phEC_M24FEP also carried the raffinose metabolism operon *rafRABDY*, a N6A type I restriction/modification system, and several pathogenicity-related genes. Phage lysate preparation and transduction assays revealed a ca. 105/ml transduction efficiency. Plaque assays confirmed the lysis capacity of *E. coli* host, however individual lysis plaques were not visible. Based on blast search in NCBI database and *in vitro* host range determination, phEC_B25FEP & phEC_M24FEP appeared restricted to *E. coli* and *Salmonella*. This Enterobacteria phage represents a novel phage genus not yet described among bacteriophages in public databases (Refseq, Genbank, VIRIDIC). Phage particles have a typical icosahedral head and a short contractile tail and carry the double-stranded DNA phage genome. Induction assays using various antibiotics as well as mitomycin C did not show any increase in transduction efficiency.

**Conclusions:** A novel family of active temperate phages carrying antimicrobial resistance genes, especially the ESBL gene *bla*CTX-M-55 was characterized in avian *E. coli* isolates. The co-localization of antimicrobial resistance genes, metabolism function, pathogenicity-related factors may increase the risk for co-selection, persistence and dissemination in food-producing animals.

## *t03 - Understanding the connection of antimicrobial resistance between animals, humans and/or the environment*

**P-005**

# Livestock as a potential reservoir of antimicrobial resistance of last resort: Genetic diversity of tigecycline-resistant *Klebsiella* spp. partially co-conferring colistin resistance

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**Background and objectives:** Multidrug-resistant (MDR) *Klebsiella* spp. are important nosocomial pathogens. Due to their high genetic adaptability to a wide range of stress responses, this member of the ESKAPE bacteria is receiving increasing attention from public health authorities as they are difficult to treat. Because of their widespread availability (humans, animals and environment), there is a need to identify sources of MDR isolates that confer resistance to clinically important antimicrobials and to prevent their spread. Here, we characterized tigecycline-resistant *Klebsiella* isolates using whole-genome sequencing (WGS) and bioinformatic analysis in Türkiye in 2022.

**Methods:** A total of forty *Klebsiella* isolates, comprising turkey ceacum samples (n=23, *K. pneumoniae*), chicken ceacum samples (n=9, *K. pneumoniae* and n=2, *K. oxytoca*) and chicken meat samples (n=7, *K. pneumoniae*), were analyzed. In addition to antimicrobial susceptibility testing (AST), their genomes were characterized by WGS with the Illumina Hiseq 2000 and Oxford Nanopore MinION platforms and subsequent bioinformatic analysis for subtyping, phylogenetic evaluation (multilocus sequence typing [MLST] and core genome MLST [cgMLST]) and risk assessment (acquired/transmissible antimicrobial resistances).

**Results:** In addition to the highly heterogeneous phenotypic resistance pattern, the isolates also showed strong genetic diversity by MLST in which eleven known sequence types (i.e., ST37, ST147, ST423, etc.) were determined for *K. pneumoniae* isolates, while all *K. oxytoca* belonged to ST145. Our results showed that the *tet*(A) gene was present in almost all *K. pneumoniae* isolates, whereas other tetracycline-encoding genes were also detected in few isolates, including *tet*(B), *tet*(D), *tet*(J) and *tet*(M). Various other acquired AMR determinants caused resistance to several classes of antimicrobials were also found. Notably, some of the isolates conferring colistin resistance also exhibited the colistin resistance gene *mcr-8*, which was found to be plasmid encoded. Among the isolates, IncFIA(HI1), repB(R1701), and IncFIB(K)-IncFII(K) were the main types of plasmids, that responsible for the phenotypic spread of colistin resistance.

**Conclusions:** The study indicates that poultry and chicken meat appear to be a potential reservoir of MDR klebsiellae. In particular, resistance to antimicrobials of last resort is of concern and must be avoided to protect public health.

## P-089

# Transmission and Exposure Risk of Airborne Antimicrobial Resistance Originating from Livestock Farming

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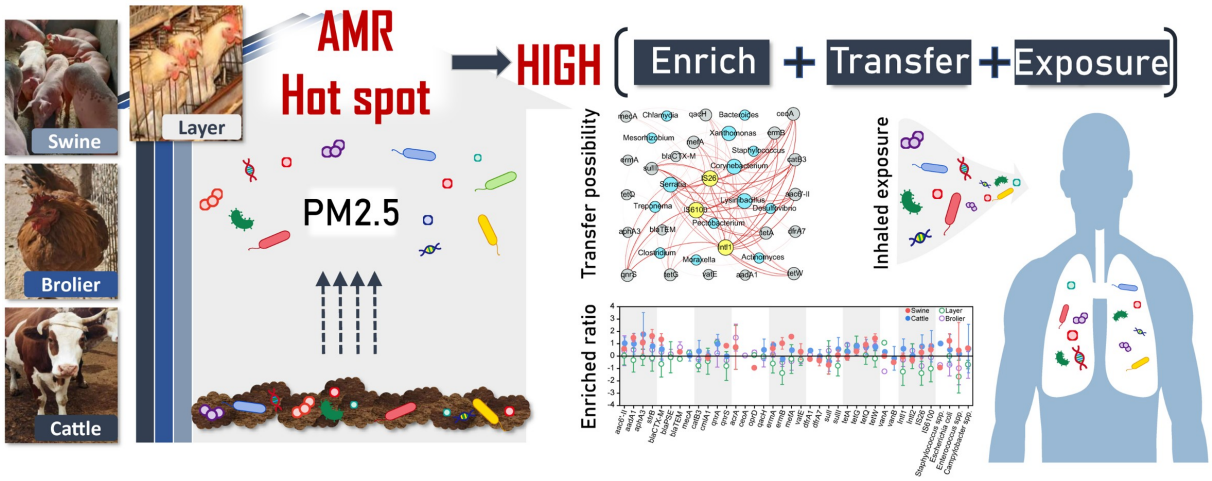
**Background and objectives:** Animal farms are the known reservoirs for environmental antimicrobial resistance (AMR). As an indispensable part of human-animal-environment loop regarding "One Health" issue, animal-related AMR has been intensively studied, which mainly focused on identifying resistomes in animal feces and their environmental dimensions in terrestrial and aquatic ecosystem. However, knowledge of AMR burden in the air around animal farms remains disproportionately limited.

**Methods:** A total of 60 samples (40 air and 20 fecal samples) were collected from swine, cattle, layer, and broiler farms in Beijing, China, with five farms visited for each animal species. Using metagenomic sequencing, digital PCR, and other techniques, we comprehensively analyzed the contamination characteristics, transmission processes, and exposure risks of ARGs and antibiotic-resistant bacteria (ARB) in the air and feces of these farms.

**Results:** The results showed that the average concentration of ARGs in farm air ranged from 102 to 104 copies/m³, with high concentrations of mobile genetic elements (MGEs) and potential pathogenic bacteria (HPBs) detected (103 copies/m³). As a significant source of airborne AMR, HPBs and ARGs in air and fecal samples exhibited significant correlations, with stronger co-occurrence of ARGs, MGEs, and HPBs in the air. Moreover, the potential transferability of ARGs in the air was significantly enhanced. The contamination characteristics and potential transferability of airborne ARGs also demonstrated species-specific patterns. Additionally, we assessed the aerosolization behavior of ARGs and ARB, finding that 70% of ARGs and 60% of ARB had a preferential aerosolization capacity, primarily influenced by animal age, stocking density, and rearing area. Farm workers may inhale approximately 104 copies of ARGs daily, exceeding other intake pathways. Furthermore, the high exposure levels of MGEs may facilitate the horizontal transfer of ARGs and pathogenic bacteria, amplifying the potential exposure risks of ARGs.

**Conclusions:** The distinguishing features of airborne AMR were revealed by comparison with that in animal feces. This confirmed that animal farms served as a hot spot for airborne antimicrobial resistance. In air environments of animal farms, the AMR genes in PM2.5 were universally enriched, especially in swine and cattle farms. The potential transfer ability of airborne ARGs was strengthened to their possible pathogenic host. Some AMR genes could be inhaled with a considerable daily intake load, which is comparable to via drinking water. Based on a description of two important AMR hotspots, this study reveals the overall profile of AMR pollution in animal farms. Current results will help to comprehensively assess the pollution status of AMR in farms, and provide a research basis for the subsequent formulation of policies and technologies to control the spread of animal AMR to the environment.

**Figure**




**P-090**

# Avian Pathogenic *Escherichia coli* in Humans and Poultry in the Czech Republic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**Background and objectives:** Extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages are one of the major causes of urinary tract infections, neonatal meningitis and sepsis. One subgroup of ExPEC is avian pathogenic *E. coli* (APEC). For many years, APEC were considered as opportunistic pathogens causing secondary bacterial infections in poultry and their virulence was underestimated, however, significant similarities have been recently observed between some strains from livestock and humans. The aim of the study is to investigate the molecular epidemiology of APEC strains from humans and poultry and to determine which specific features they share.

**Methods:** We have collected human isolates from urine and stool and veterinary isolates from poultry. Human isolates were collected in clinical laboratories as susceptible and resistant to cefotaxime (2

mg/L). For veterinary isolates, *E. coli* strains were selected using MacConkey agar (MCA) without antibiotics and in parallel on MCA with cefotaxime (2 mg/L) to detect cephalosporin-resistant strains. The isolates were identified by MALDI-TOF mass spectrometry followed by selection of APEC strains by multiplex PCR targeting five genes associated with this pathotype (*iroN, hlyF, iutA, ompT*, *iss)*. A representative set of APEC strains has been selected for whole-genome sequencing.

**Results:** We obtained 1566 human *E. coli* isolates from urine (n=786) and stool (n=798) and after PCR screening 269 (17.2%) APEC strains were identified. Urine-derived isolates predominated (64.3%), with a higher prevalence among female patients (65.4%). More than 70% of these isolates were sensitive to cefotaxime. From the veterinary sector, we were able to collect a total of 585 samples derived from chicken fresh meat (n=181), broiler appendix (n=156), faeces (n=187), cuffs and crate swabs (n=61). In this set, we identified 191 *E. coli* isolates and n=72 (37.7%) of them were confirmed as APEC. The majority of the strains came from meat (n=27) and poultry faeces (n=21) and were susceptible to cefotaxime.

**Conclusions:** A significant proportion of APEC strains were detected among *E. coli* isolates from humans and poultry. APEC strains were found to be more prevalent in female urine samples, fresh poultry meat and poultry faeces. Most of the identified APEC isolates were found to be susceptible to cefotaxime, which does not exclude their resistance to other clinically relevant antibiotics. Therefore, analysis of whole genome sequences is necessary to better understand the molecular epidemiology and antimicrobial resistance of APEC strains. Advanced analyses will enable the comparison of specific traits shared among these strains and help uncover the driving forces behind their spread.

## P-091

# Quaternary Ammonium Compounds: A New Driver and Hidden Threat for mcr-1 Prevalence in Hospital Wastewater and Human Feces

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**Background and objectives:** The emergence of mobile colistin resistance gene *mcr-1* has attracted global attention. The prevalence of *mcr-1*-positive *Escherichia coli* (MCRPEC) from both healthy humans and inpatients was largely decreased after banning colistin as animal growth promoter in China. However, the prevalence of MCRPEC in hospital environment and the relationship between disinfectants and *mcr-1* remain unclear.

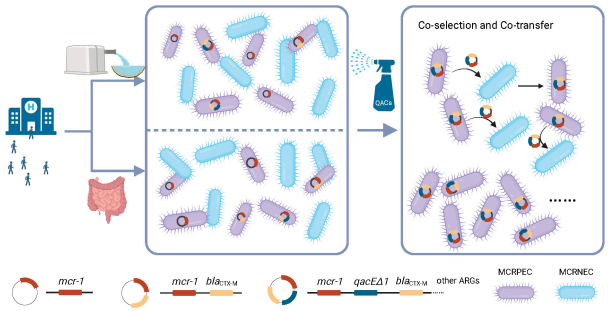
**Methods:** MCRPEC and *mcr-1*-negative *E. coli* (MCRNEC) were collected from human feces hospital wastewater. Genetic relationships among human- and wastewater-derived MCRPEC and co-occurrence of *mcr-1* with other antimicrobial resistance genes and disinfectant resistance genes (DRGs) were analyzed using whole genome sequencing based molecular methods. The disinfectants—quaternary ammonium compounds (QACs), ortho-phthalaldehyde (OPA), and povidone–iodine (PVP–I) were used to evaluate the potential co-select and co-transfer for *mcr-1*-positive plasmid.

**Results:** We found that the prevalence of MCRPEC was low in human feces (4.6%, 71/1532) but high in hospital wastewater (50.0%, 27/54). *mcr-1* was predominantly located on IncI2 (63.0% in wastewater, 62.0% in feces) and IncHI2 plasmids (18.5% in wastewater, 21.1% in feces). High genetic similarity of the *mcr-1* context and its carrying plasmids was observed in human and wastewater

MCRPEC, with several isolates clustering together. The coexistence of the ESBL gene *bla*CTX-M with *mcr-1* in 19.7% of IncI2 plasmids. Notably, 60.0% of IncHI2 plasmids exhibited co-occurrence of *mcr-1* with DRG *qacEΔ1*, conferring resistance to QACs. We revealed that QACs, rather than the other two types of disinfectants OPA or PVP–I, select for plasmids carrying both *qacEΔ1* and *mcr-1* and elevate their conjugative transfer frequency.

**Conclusions:** we revealed the transmission of *mcr-1* and MCRPEC between humans and hospital environments, and the critical role of disinfectants in selecting for *mcr-1* and other ARGs. These findings emphasize the need for monitoring of both *mcr-1* and *qacEΔ1* in MCRPEC and the strategic management of disinfectants used to mitigate the spread of colistin resistance in healthcare settings.

**Figure**




## P-092

# From River to Clams: A longitudinal study on the factors driving the dissemination of antimicrobial resistance genes in estuarine environments

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**Background and objectives:** The spread of antimicrobial resistance between animals and humans is well-known; however, the role of the environment, particularly in estuarine ecosystems, remains less explored. Estuarine waters, downstream of catchments with human activities may be polluted by fecal bacteria carrying antibiotic-resistance genes (ARGs) and chemicals such as heavy metals, biocides or disinfectants. Due to their high filtration capacity, clams accumulate urban and agricultural bacteria, ARGs, and pollutants. Clam-related outbreaks have been reported, and the presence of antibiotic-resistant bacteria or ARGs in clams may pose additional risks to human health. This longitudinal study investigated the microbiome, resistome, and environmental factors driving the spread of ARGs in estuary to better understand their prevalence and dynamics.

**Methods:** The presence of ARGs and integrons was assessed using high-throughput microfluidic qPCR (targeting 45 ARGs from nine antibiotic families and two integrons) in river, estuarine water, surface sediments and clams, from an anthropized site in the Bay of Brest (France), over a 14-month period (n = 258). Simultaneously, hydrological data (river flows), physico-chemical parameters (temperature, salinity, conductivity, nutrients...), fecal bacterial indicator (*E. coli*), and other pollutants (biocides, toxic metals, and antibiotics) were measured. Bacterial communities were analyzed using

V3-V4 16S rRNA gene amplicon sequencing processed using SAMBA (v4.0.1). Correlation analyses were performed to explore the relationships between ARGs, bacterial diversity, environmental factors and pollutant concentrations.

**Results:** Fifteen ARGs conferring resistance to eight antibiotic classes, including aminoglycosides (*strA*, *strB*, *ant(3')Ia*), β-lactams (*blaTEM*, *blaSHV*), folic acid synthesis inhibitors (*sul1*, *sul2*, *dfrA1*), macrolides (*mphB*, *mphE*, *ermB*), phenicols (*floR*), polymyxins (*mcr4*) and tetracyclines (*tetA, tetM*), as well as an integron (*int1*), were sporadically detected in clams during the monthly monitoring. These ARGs were also found at higher frequencies in waters and/or sediments, with other genes conferring resistance to β-lactams (*blaCTX-M1, blaPER1, blaOXA23, blaOXA48, blaOXA51, blaOXA58, blaCMY1-3*), fluoroquinolones (*qnrA, qnrB1, qnrS*), folic acid synthesis inhibitors (*sul3, dfrA12*, *dfrA17*) and macrolides (*mph*A). Bacterial community analysis showed that 24,1% of bacterial genera were shared between clams, sediments, and waters, representing 75% of the total relative abundance. Correlation analysis between the presence of ARGs and bacterial genera revealed significant associations (Student's t-test p-value < 0.01) with strong correlations (Pearson's r > 0.8). Notably, associations were identified between *blaCTX-M1*, *blaSHV*, *mphA,* and *sul3* in river water, and *dfrA1* and *sul2* in estuarine water with *Arcobacter,* a ubiquitous and pathogen-related genus. This genus was detected in both river and estuarine waters, as well as in clams, with its prevalence significantly increasing after heavy rainfall (8.6–18.3 mm) (Pearson"s r = 0.94, p < 0.001 in river water; r = 0.88, p < 0.001 in estuarine water and r=0,48, p <0,01 in clams). In the river, its occurrence was strongly correlated with agricultural pollutants, including Metolachlor ESA (r = 0.59, p < 0.05) and Metolachlor OXA (r = 0.93, p < 0.001). In estuarine water, Arcobacter was associated with agricultural pollutants, such as Metolachlor NOA and Metolachlor OXA (r = 0.75, p < 0.01), and fecal contamination *(E. coli* concentrations) (r = 0.87, p < 0.001).

**Conclusions:** These results highlight the presence of ARGs in estuarine environments, including in clams, and reveal strong correlations between ARGs and pathogen-related genera. These findings provide valuable insights into the potential links between environmental, human, and animal health, enhancing our understanding of the dynamics and dissemination of antimicrobial resistance in estuarine ecosystems.

## P-093

# Genomic comparison of the Czech and global population of Extended-Spectrum Beta-Lactamase-encoding Extraintestinal Pathogenic *Escherichia coli* lineage ST69

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**Background and objectives:** High-risk clones of extraintestinal pathogenic *Escherichia coli* (ExPEC) producing extended-spectrum beta-lactamases (ESBL) pose a significant threat to both human and animal health. One such clone is *E. coli* ST69, which is widely distributed but the features behind its success are not yet fully understood. In this study, we analysed 160 *E. coli* ST69 isolates from the Czech Republic and compared them to 2,892 publicly available *E. coli* ST69 genomes.

**Methods:** All ST69 genomes were analysed for the content of antibiotic resistance genes (ARGs), plasmids and other genetic determinants. The phylogenetic and GWAS (genome-wide association study) analysis were performed using Prokka, Bakta, Roary, RAxML, and Scoary tools.

**Results:** Czech *E. coli* ST69 collection consisted of isolates of human (n=100), environmental (n=46) and animal origin (n=14). Czech isolates were classified to phylogroup D with O15:H18 (53/160) and O17:H18 (35/160) serotypes being the most common. Regarding the classes of ARGs, resistance to beta-lactams, sulfonamides and aminoglycosides being the most prevalent across sources. Genes conferring ESBL production were detected in 75 isolates (47%, n=160), and were mostly represented by $bla_{\text{CTX-M-15}}$ (40/160). At least one plasmid replicon was found in 151 isolates, with IncFIB predominating (118/160). Similar trends in serotype distribution, antibiotic resistance and plasmid prevalence were observed among the global ST69 collection.

Phylogenetic analysis of Czech collection showed several related clusters (≤ 100 SNPs) with isolates of different origin, mainly consisting of environmental and human isolates. The GWAS analysis revealed the presence of chromosomally encoded operon of unknown function, probably involved in ribose metabolism, however *E. coli* already harbours genes for ribose metabolism. While this operon was detected in 13 Czech *E. coli* O45:H4 (8.2%, n=160), it was found only in 24 isolates of the global ST69 collection (0.8 %, n=2,892). The presence of this operon was detected only in three genomes of non-ST69 *E. coli* in GenBank compared to 45 and 25 hits in *K. pneumoniae* and *K. oxytoca,* respectively.

**Conclusions:** The *E. coli* ST69 lineage displays widespread multi-drug resistance, with a high prevalence of ESBL production and plasmid associations across various sources, emphasizing their significance in a One Health context. Unique genetic features, such metabolic operons, highlight the importance of deep genomic analysis to understand the global threat posed by ExPEC strains.

## P-094

# A Pilot Study to Identify the Prevalence of Antimicrobial-Resistant Bacteria in Raw Pet Food Ingredients in the UK in 2024

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**Background and objectives:** Raw pet food is growing increasingly popular as part of companion animal diets due to the potential benefits to fur condition, dental hygiene and digestion. However, raw pet food can pose a threat to animal and human health due to the risk of contamination with food-borne pathogens, such as *Salmonella*, and antimicrobial-resistant (AMR) bacteria. Therefore, as part of the PATH-SAFE (Pathogen Surveillance in Agriculture, Food and Environment) program, which aims to develop a better national surveillance system for the monitoring and tracking of foodborne disease and AMR in the environment and agri-food system, an exploratory pilot study was designed to identify the prevalence of AMR bacteria and food-borne pathogens in a limited number of imported raw meat ingredients destined for production of raw pet food products in the UK.

**Methods:** Raw pet food manufacturers and UK ports were invited to participate in a four-month pilot study (August to November 2024) to identify the prevalence of *Escherichia coli* (including indicator *E. coli*, cefotaxime [CTX]- and carbapenem-resistant *E. coli*), *Enterococcus faecalis*, *Enterococcus faecium* and *Salmonella* spp. in raw meat ingredients. Participants were given questionnaires to complete regarding the uniqueness of the sample batches, country of export as well as the country of

freezing (if samples were frozen). Raw meat ingredients were screened for the presence of bacteria, according to EU protocols for the harmonised monitoring of AMR bacteria (Commission Implementing Decision [EU] 2020/1729), using selective agar (MacConkey and MacConkey + cefotaxime for isolation of *Escherichia* coli; Modified Semisolid Rappaport Vassiliadis (MSRV) agar and Rambach for *Salmonella* spp. as well as Slanetz and Bartley agar for *Enterococcus* spp.). Antimicrobial susceptibility testing was performed using the broth microdilution method (using Sensititre™ plates EUVSEC 3 and EUVSEC 2 with EFSA ECOFFs) and AMR genes were identified using whole-genome sequencing (WGS; Illumina NextSeq 500/550 and Oxford Nanopore Technologies) and the APHA SeqFinder V1 pipeline.

**Results:** Through analysis of the results, we will determine the prevalence of AMR- and food-borne bacteria in the sampled raw meat ingredients

**Conclusions:** These results will subsequently support the identification of any potential risks related to importing raw pet food ingredients into the UK.

## P-095

# Evaluating colistin use in veterinary medicine: A narrative literature review

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**Background and objectives:** Colistin plays a pivotal role in human and veterinary medicine. It is considered the last-line agent to treat infections caused by gram-negative bacteria. Studies have shown that there is an increasing rate of colistin resistance worldwide.

Due to the importance of colistin, several regulatory authorities set different legislations to tackle the problem of colistin resistance. It is proven that resistance transmits from animals to humans via consuming food products from animals.

A narrative literature review of previous research and studies was conducted. This review aims to identify the impact of colistin use in veterinary medicine and propose future research directions in this area.

**Methods:** A comprehensive literature review of studies assessing colistin use in human and veterinary medicine was conducted to analyze findings in a narrative scheme. Two electronic databases were used for this search: ScienceDirect and Google Scholar.

Articles and studies that were conducted in the time frame between 2000 to 09-2024 were included. The selection criteria in this search was using colistin in food-producing animals in any country. Articles using colistin in humans only were excluded. Also, articles that utilized antimicrobial agents other than colistin were excluded from this review.

**Results:** Antimicrobial resistance has raised recently due to the unregulated use of highly important antibiotics such as colistin. Various studies indicate high level of colistin resistance in different countries worldwide. This resistance is mostly transferred from animals to humans. The Colistin resistance genes were found in specimens of such animals. A study involving data from around 26 European countries shows a statistically significant correlation between the use of colistin in food-producing animals and the development of colisitin resistance.

Establishing guidelines on the use of colistin in veterinary medicine is crucial to reduce the spread of resistance in humans and animals as well. Additional precautions should be followed to decrease the improper use of antibiotics in animals such as vaccination programs and applying proper hygiene practices.

The main recommendation is that colistin sales for use in animals should be reduced to the minimum feasible. In addition, the use of colistin should be evaluated case by case depending on the presence of susceptible bacteria.

**Conclusions:** Colistin plays a substantial role in treating bacterial infections in humans and animals. It is of high importance to set rules and regulations to prevent the inappropriate use of critically important antibiotics such as colistin. It is well known in the literature that colistin resistance can transfer from animals to humans via consuming food such as meat, eggs, and milk. Thus, regulating the use of colisitin in veterinary medicine is essential to combat the spread of antimicrobial resistance. In many countries, multiple regulations were established to prevent the misuse of antibiotics in animals such as; preventing the use of important antibiotics as a growth promoter, preventing the use of antibiotics as a metaphylactic agent, and preventing the use of highly important antibiotics such as colistin to treat herds. Instead, a decision to use colistin was made case by case by a responsible veterinarian.

## P-096

# Genomic Insights into Plasmids of Multidrug-Resistant *Shigella*

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**Background and objectives:** *Shigella* is a causative agent of various infections in humans and primates mainly associated with dysentery which can lead to death. Virulence and antibiotic resistance of *Shigella* spp. is associated with plasmids of F-type incompatibility group (IncF-type). While the virulence plasmid is strictly tied to *Shigella* spp., plasmids carrying multiple antibiotic resistance genes (ARGs) can circulate among other *Enterobacteriaceae* in different hosts and environments.

**Methods:** From January 2021 to December 2023, twelve stool samples from patients in Czech Republic were collected. Five *Shigella flexneri* and six *S. sonnei* isolates were selected for Illumina short-read sequencing. Based on the genetic content (plasmids, ARGs, serotyping, phylogenetics), four representatives were selected for long-read sequencing on MinION platform to assess the plasmids circulating *Shigella* spp. in Czech Republic. The genomes of four representatives were *de novo* assembled using Unicycler. The obtained complete plasmids were classified for plasmid replicons, *Shigella* plasmid presence and IncF-type replicon sequence type using ABRicate with PlasmidFinder database, ShigaTyper and pMLST tools. ARGs and VAGs carried by plasmids were identified by ABRicate with databases ResFinder and VFDB, respectively. Plasmids were automatically annotated by Bakta and compared using clinker.

**Results:** The analyzed isolates of *S. sonnei* from Czech Republic belonged to a lineage 3 and *S. flexneri* were identified as serotype 2a and 3a. Ten out of eleven sequenced isolates carried multiple ARGs and plasmids. Eight isolates carried a large virulence IncF-type plasmids of RST F27:A-:B- specific to *Shigella*. Moreover, most of the isolates harbored another IncF-type plasmid carrying ARGs. The most prevalent resistance plasmid was F2:A-:B- carrying $bla_{\text{CTX-M-15}}$ conferring resistance to cephalosporins. This plasmid was previously identified in animals, waste waters and other environments.

**Conclusions:** The *Shigella* isolates from patients from Czech Republic carried the common virulence F27:A-:B- plasmid of *Shigella* spp. The presence of the resistance plasmids in *Shigella* emphasizes the crucial role of plasmids in the adaptation and survival of *Shigella*. These plasmids were previously detected in diverse environments, highlighting their potential for the spread outside of human hosts.

**P-097**

# Distribution and transmission of apramycin-resistant *Escherichia coli* from humans and animal-producing sectors: a One Health study

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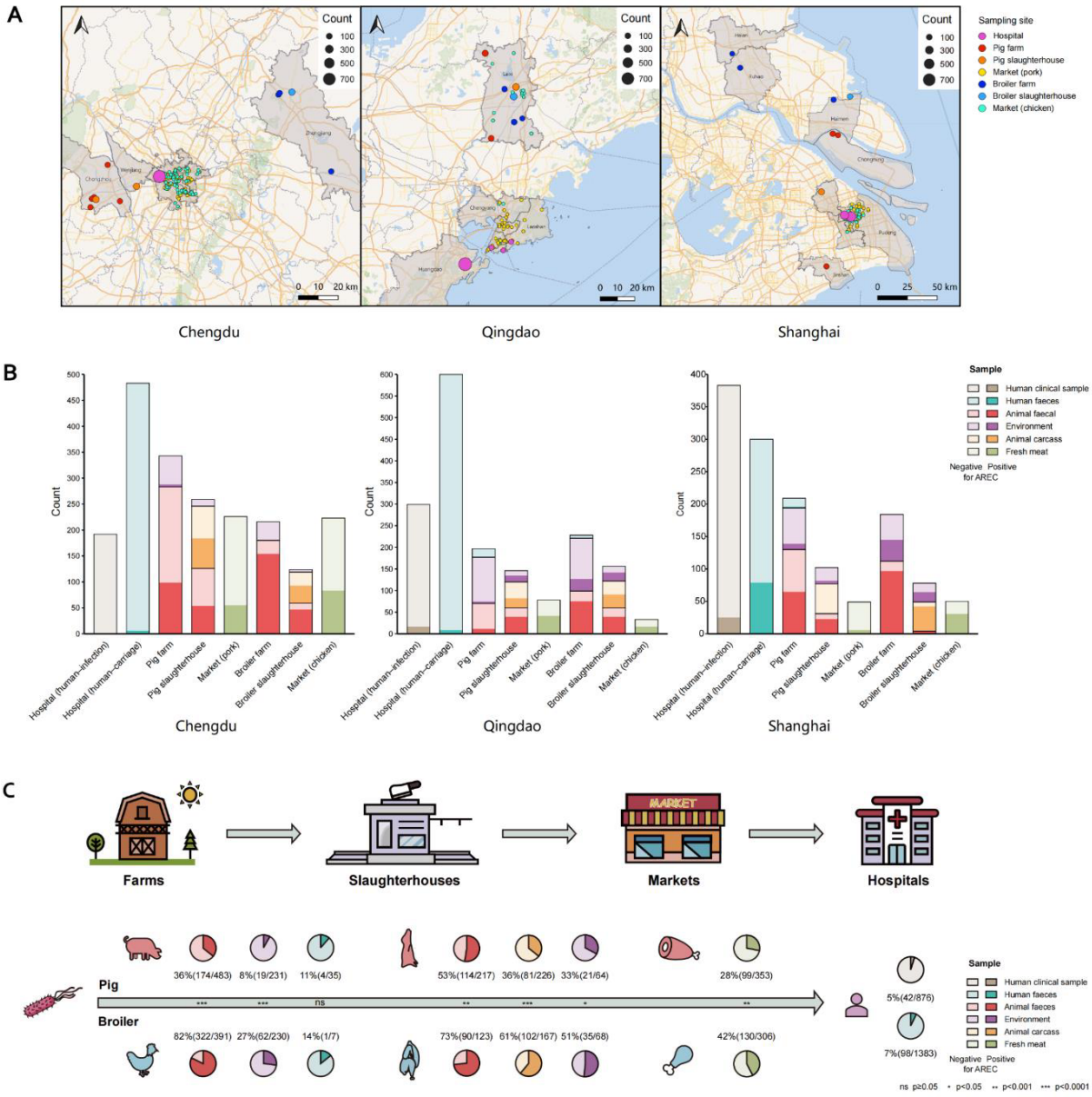
**Background and objectives:** Apramycin, an aminoglycoside antibiotic used exclusively in veterinary medicine, has gained attention for its potential clinical use due to its low toxicity and effectiveness against multidrug-resistant bacteria. However, the dynamics of resistance across One Health interfaces—spanning human, animal, and environmental sectors—remain poorly understood. This study aimed to investigate the prevalence, resistance mechanisms, and transmission patterns of apramycin-resistant *Escherichia coli* (AREC) across these interfaces.

**Methods:** From 2020 to 2023, a total of 5160 non-duplicate samples were collected in Chengdu, Qingdao, and Shanghai, China, from hospitals, broiler and pig farms, slaughterhouses, and markets. Samples included animal feces, carcasses, fresh meat, environmental sources, human feces, and clinical specimens. AREC isolates were identified and analyzed for resistance genes, with 742 isolates subjected to genomic analysis and 66 representative isolates undergoing long-read sequencing to assess plasmid structures. Temporal trends in resistance prevalence were also evaluated.

**Results:** Of the 5160 samples, 1394 AREC isolates were identified, with the highest detection rates in animal feces (58%, 700/1214), followed by animal carcasses (47%, 183/393), fresh meat (35%, 229/659), environmental samples (23%, 127/592), human feces (7%, 103/1425), and clinical samples (5%, 42/876). Detection rates were notably higher in broiler-producing chains (57%, 742/1292) than in pig-producing chains (32%, 512/1609). Nearly all AREC isolates (99.7%, 1390/1394) carried the *aac(3)-IV* gene, conferring resistance to apramycin, gentamicin, and tobramycin. Genomic analysis revealed sporadic clonal transmission between animals and humans in Qingdao and Shanghai. Long-read sequencing showed that *aac(3)-IV* genes were predominantly located on structurally conserved IncHI2/IncHI2A plasmids across sources. A marked increase in *aac(3)-IV* prevalence in livestock-associated *E. coli* was observed following the introduction of apramycin in China.

**Conclusions:** This study demonstrates the rapid, plasmid-driven spread of apramycin resistance across One Health interfaces, with significant dissemination from livestock to humans and the environment. The findings underscore the urgent need for improved stewardship of apramycin use in veterinary settings and caution against its repurposing for human clinical applications without addressing resistance risks.

## Figure


A
Count
100
300
500
700
Sampling site
Hospital
Pig farm
Pig slaughterhouse
Market (pork)
Broiler farm
Broiler slaughterhouse
Market (chicken)
0 10 20 km
0 25 50 km
Chengdu
Qingdao
Shanghai
B
Count
Sample
Human clinical sample
Human faeces
Animal faecal
Environment
Animal carcass
Fresh meat
Negative Positive
for AREC
Hospital (human-infection)
Hospital (human-carriage)
Pig farm
Pig slaughterhouse
Market (pork)
Broiler farm
Broiler slaughterhouse
Market (chicken)
Chengdu
Qingdao
Shanghai
C
Farms
Slaughterhouses
Markets
Hospitals
Pig
Broiler
36%(174/483)
8%(19/231)
11%(4/35)
53%(114/217)
36%(81/226)
33%(21/64)
28%(99/353)
82%(322/391)
27%(62/230)
14%(1/7)
73%(90/123)
61%(102/167)
51%(35/68)
42%(130/306)
ns
5%(42/876)
7%(98/1383)
Sample
Human clinical sample
Human faeces
Animal faeces
Environment
Animal carcass
Fresh meat
Negative Positive
for AREC
ns p≥0.05 * p<0.05 ** p<0.001 *** p<0.0001

**P-098**

# Evaluation of The Use of Antibiotics in The Veterinary Sector in Georgia in 2021-2023

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**Background and objectives:** The National Action Plan was developed in Georgia in 2017 to define a unified policy against antimicrobial resistance [1]. This strategy is implemented in a coordinated manner by the Ministry of Health (MOH) and the Ministry of Environmental Protection and Agriculture (MEPA). We aimed to evaluate the national antimicrobial use (AMU) in the veterinary sector using different data sources and if antimicrobials were used for growth promotion.

**Methods:** We estimated AMU from 2021 to 2023 by (i) collecting official AMU data from government sources including the National Statistics Office of Georgia (NSOG) and the National Food Agency (NFA), (ii) sending a questionnaire on antimicrobial sales to 12 major distributors of veterinary products, and (iii) collecting AMU data from two large commercial poultry farms in Georgia.

**Results:** Data from the NSOG provided an overall estimate of the amount spent on imported and exported antibiotics for the following antimicrobial classes: penicillins, tetracyclines, sulphonamides, tetracyclines, chloramphenicol, erythromycins, fluoroquinolones, their derivatives, and antibiotics belonging to other classes. The largest amount was spent on the import of tetracyclines. There was no indication on volumes nor if antibiotics were intended for humans or animals. According to data from the NFA, the quantities of antibiotics sold in Georgia were 11,300 kg, 7,052 kg, and 9,927 kg in 2021, 2022 and 2023 respectively; however, reporting on antibiotic sales from distributors is not mandatory.

Only one distributor provided detailed information in response to our survey. It sold a total of 148 tons of antibiotics from all classes in 2020, 91 tons in 2021, and 79 tons in 2022, i.e. volumes that are significantly higher than the national estimates from NFA. Neither data from NFA nor from the distributor were stratified by animal species. Farm 1 used approximately 1600 kg/year and Farm 2 used approximately 125 kg/year of tetracyclines for growth promotion alone.

**Conclusions:** Our results suggest that the data that is based on voluntarily reporting to NFA, and indirectly to the World Animal Health Organization, is largely underestimated. We recommend improving the national monitoring system for AMU in the animal sector in Georgia, by making it mandatory for distributors to provide their annual antimicrobial sales data. In addition, a representative farm-level study on AMU in Georgia is necessary to better understand how antibiotics are used for the main animal species.

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**P-099**

# Monitoring of antimicrobial resistance in the interface between domestic animals, wildlife, and the environment

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**Background and objectives:** The establishment of the Quadripartite partnership in March 2022, has reaffirmed the importance of the environmental sector in One Health [1]. The Quadripartite considers One Health as the main approach for addressing complex health challenges such as antimicrobial resistance (AMR). In its latest call to action, the Quadripartite urged stakeholders to focus on a number of priority actions, among which to strengthen and sustain prevention of health threats at source, in order to reduce the risk of zoonotic spillover. The Quadripartite"s One Health Joint Plan of Action extensively addresses the possible spillover of AMR between food-producing animals, humans and the environment, and recognizes the role of the interface between domestic animals and wildlife in the emergence of zoonotic epidemics [1].

Denmark is internationally recognized for a long-lasting successful intersectoral collaboration for the monitoring of AMR in animals, food and humans – the DANMAP program. In a recent evaluation of its One Health scope, DANMAP scored lowest in the target of coverage/transdisciplinary, due to the lack of representation of the environmental sector in its program [2]. The monitoring of AMR in the environment is crucial to understand the dynamics of AMR dissemination outside of clinical, veterinary and farm-to-fork settings [3]. It can serve both the purposes of assessing the dissemination of already resistant bacteria, and of studying how the environment acts as a source of new AMR hazards [3]

Research on the environmental monitoring of AMR of anthropogenic origin is in its infancy, with the majority of the existing studies having focused on farm waste or wastewater. However, wildlife is known to have a role in the spread of AMR and has been increasingly hypothesized as a useful marker for environmental pollution with antimicrobial resistance [4].

**Hypothesis:** Terrestrial animals participate in the spread of resistance between food producing animals and the surrounding ecosystem. Mussels are successful bioindicators for environmental spread of AMR from the terrestrial to the aquatic environment. *E. coli* can be used as indicator for future environmental surveillance of AMR.

**Methods:** Fecal samples were collected from septic tanks from pig farms selected within a 5 km radius of the Limfjord straight in western Denmark. Fecal content from wildlife and whole mussels were collected from the surrounding area. *E. coli* was isolated from all samples on selective media (MacConkey) and DNA was isolated followed by whole genome sequencing and phenotypic testing (broth microdilution). All samples had geolocation. Phylogenetic analysis will be performed to investigate the genomic relationship between the *E. coli* isolates, and the pathway of AMR environmental spread will be modelledutilizing GIS software.

**Results:** Preliminary results will be available at the conference.

**Conclusions:** Preliminary conclusions will be available at the conference.

**References:**

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**P-100**

# One Health approach to assess antimicrobial resistance to cephalosporins and carbapenems in sub-Saharan Africa

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**Background and objectives:** Resistance to high-priority antibiotics is considered a major global health threat. As humans, animals and the environment are closely interlinked, a One Health approach is considered essential to understand the patterns and drivers of AMR in order to identify appropriate intervention points. While high-income countries are establishing AMR surveillance at least for humans and livestock, low- and middle-income countries face critical challenges in establishing a surveillance system.

**Methods:** The ADAPT project aims to establish a true One Health sampling framework to support AMR management in seven sub-Saharan countries by strengthening local capacity to identify key transmission pathways. Using a One Health approach, researchers from eight participating countries are investigating AMR links between humans, animals, and the environment.

**Results:** A harmonised sampling scheme has been agreed. In each country, 500 samples will be collected, including cattle and chicken (caecal contents at slaughter), vegetables at retail markets and wastewater samples. This will be supplemented by 200 available isolates from human clinical cases. Animals, food and environmental samples are tested for extended-spectrum beta-lactamase producing *E. coli* (ESBL-EC), carbapenem-resistant *E. coli* (CREC) and Salmonella using harmonised selective methods. Antibiotic susceptibility testing (AST) using agar diffusion includes a harmonised set of antimicrobials as well as confirmation of ESBL and CR type. Agreed common protocols, reference strains and proficiency testing ensure valid results. Whole genome sequencing is performed on a subset of isolates.

**Conclusions:** For the first time, a surveillance system with standardised protocols and procedures has been established. Early results already underscore the urgent need for effective AMR surveillance and intervention in the sub-Saharan countries. Strengthening local capacity within a One Health framework is essential to reduce the spread of AMR and improve public health in sub-Saharan Africa.

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## P-101

# Antibiotic Use and Occurrence of Fluoroquinolones Residues in Slaughtered Chickens at Live Bird Markets, Abuja, Nigeria

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**Background and objectives:** Antibiotic are normally used in poultry industry to improve health, reduce incidence of diseases and enhance productivity. Antibiotic drugs given to poultry birds have been shown to be retained in animal tissue, especially when the correct dosage is not adhered to and when such birds are slaughtered without the observance of withdrawal period which then becomes harmful to consumers. Enrofloxacin is the most common fluoroquinolones used in veterinary practice; its major metabolite, ciprofloxacin is commonly used in human medicine. Administration of Enrofloxacin in poultry without the observance of withdrawal period has been shown to lead to ineffective treatment with ciprofloxacin in human.The study was carried to determine the antibiotic use and occurrence of fluoroquinolones residue in slaughtered chickens at live bird markets.

**Methods:** We conducted a cross sectional study in 8 live bird markets, Abuja, using multi-stage sampling method. Questionnaire was randomly administered to 280 chicken sellers, with sub-section on socio-demographics of the sellers, poultry information and information on antibiotic drugs use. Chicken liver samples (271) were collected for testing for residues using microbiology inhibition and a confirmatory test for fluoroquinolones residues using Maxsignal fluoroquinolones ELISA test kit.

**Results:** The result showed that, of the 271 chicken liver samples tested, 72 (25.71%) were positive for antibiotic drug residues, among which 21 (29.71%) were found to be positive for fluoroquinolones. Among the chicken sellers interviewed, 86.8% o had never attended any training on poultry management, about 89% use antibiotic drugs regularly among whom 96% use it for prophylaxis. All of them (100%) don"t keep records of antibiotic use. Only 2% are aware of withdrawal period while 99.3% are unaware of the harmful effects of antibiotic drugs residues to humans. About 30% sell their sick birds while 21% slaughter them. Source of the drugs were 60% from street vendors and 38.6% from veterinary stores. About 60% of prescriptions are done by the retailers.

**Conclusions:** Use of antibiotic drugs by chicken sellers at live bird markets is very rampant, uncontrolled and widespread. Fluoroquinolones happen to be among the drugs of choice being used. There is a considerably high rate of ignorance among chicken sellers regarding the appropriate use of antibiotic drugs. We recommended for intensive enlightenment campaign on the dangers of uncontrolled antibiotic use among chicken sellers.

## P-102

# Metagenomics approach for investigating AMR circulation between animals and their environment at provincial scale in Cambodia

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**Background and objectives:** 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 are reported. Cambodia is also confronted with AMR where 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 among humans, animals, and the environment in Cambodia. This information is nevertheless essential for the establishment of effective control strategies. Our aim is to study, in a "One Health" approach, the microbiome and its associated resistome present in the environment and animals of patients infected with multi-drug resistant *Enterobacteriaceae*. The research was conducted using metagenomics sequencing strategy and through the extensive use of bioinformatics workflows.

**Methods:** Patient samples were collected at the provincial hospital of Battambang. Environmental samples (e.g., soil, wastewater, drinking water), oral/rectal swab samples from animals (chickens, pigs, ducks, dogs, rodents) and food samples were collected at the patients" households. In total, 750 samples were collected and similar samples from a given household were pooled per host type to result in 469 metagenomes that were sequenced on Illumina NovaSeq instrument. The resulting reads were submitted to the nf-core/mag workflow for metagenomics analysis and MAGs (Metagenome Assembled Genomes) reconstruction. Separately, ARGs were detected at 3 different levels: reads, assemblies and MAGs, using a combination of tools. The taxonomic diversity was explored both at reads and MAGs levels.

**Results:** At the reads level, the taxonomic classification and the resistomes analyses, *i.e.* the ARGs profiles of the metagenomes, both revealed a clustering by sample type and not necessarily a clustering by household. The resistomes detected at the assembly level showed consistent tendency with a clustering of ARGs profiles according to the sample type. The most abundant resistance genes detected were the genes conferring resistance to antibiotic classes like tetracycline, beta-lactam, fosfomycin, streptomycin and lincosamide. In term of taxonomy, at the reads level, the most represented phyla were the Enterobacteriaceae and the Staphylococcaceae, particularly in metagenomes originating from animals, but also from food samples. The preliminary analysis of the MAGs showed a total number of 8,209 MAGs across 453 samples analysed, with an average of 18 good quality MAGs per sample, a minimum of 2 and a maximum of 67.

**Conclusions:** This metagenomics large-scale study at the level of Battambang Province in Cambodia showed high level of resistance in term of ARGs quantity, especially for ARGs conferring resistance to antibiotics of public health interest. This is particularly true for samples from animals and food, which suggests a higher concentration of resistant bacteria in these compartments. A more in-depth

exploration of the MAGs is ongoing, and will allow us to unravel the circulation and potential transmission of resistant bacterial species between animals and their environment within and between households.

**P-103**

# Quantitative assessment of antimicrobial resistance in dairy cattle using residue analysis, metagenomics and ddPCR

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**Background and objectives:** The spread of antimicrobial resistance (AMR) in animal farming represents a significant public health issue, especially due to the role of mobile antimicrobial resistance genes (MARGs) in its dissemination [1]. The combined use of antibiotics, biocides and dietary supplements containing heavy metal, such as copper and zinc, in livestock production may contribute to the selection and co-selection of MARGs in microbial communities[2] residing the farm environment. This study aimed to quantitatively assess MARG selection and dissemination in dairy cattle farming, focusing on their potential transmission routes within and outside the farm environment.

**Methods:** A multi-approach strategy was applied, including the collection of different matrices such as faeces, feed, colostrum and milk samples from dairy farms, the quantification of antimicrobial and metal residues through chromatographic and mass spectrometry techniques, metagenomic shotgun sequencing to determine the abundance and diversity of MARGs, droplet digital PCR (ddPCR) for absolute quantification of selected resistance genes and co-occurrence analysis to assess the association between ARGs and mobile genetic elements (MGEs).

**Results:** The highest concentrations of antimicrobial and metal residues were detected in faeces and feed, suggesting them as potential sources of selection pressure. The quantification of resistance genes through metagenomic shotgun sequencing identified a total of 719 different antibiotic resistance genes, conferring resistance to different antibiotic classes, including β-lactams, aminoglycosides, tetracyclines, macrolides, fluoroquinolones, sulfonamides, glycopeptides, and several others, as well as disinfectants and antiseptics. ddPCR results provided precise quantification of a panel of these genes ( such as blaTEM, blaCTXM, qnrS, sul2, tetA, ermB, vanA), with absolute abundances ranging from 56,1 copies/uL (blaTEM in colostrum) to 41700 copies/uL (ermB in calf faeces). The higher concentrations were observed for ermB, blaTEM, tetB and sul2, particularly in faeces. The presence of ARGs in colostrum and milk confirmed their transmission along the food chain. The identification of contig shared between ARGs and MGEs suggests a potential for horizontal gene transfer, highlighting their possible dissemination routes.

**Conclusions:** These findings highlight the complexity of AMR dissemination in dairy farms and the importance of integrated surveillance to monitor antimicrobial use and its consequences. Quantifying antimicrobial residues and resistance genes provides valuable insight into the selective pressure driving ARG selection. A One Health approach that integrate farm management optimization, alternative therapeutic strategies (such as probiotics) and education programs on prudent antimicrobial use is crucial for mitigating ARG occurrence in animal farming.

## *t04 - Roles of the environment in resistance evolution and transmission*

**P-105**

# Impacts of wastewater treatment and the exposome on antibiotic resistance gene dynamics: Insights from Guadeloupe, a French Caribbean Island

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**Background and objectives:** Wastewater treatment plants (WWTPs) are global hotspots for the dissemination of antibiotic resistance genes (ARGs), primarily driven by different anthropogenic activities. Chemical compounds commonly found in wastewater, including antibiotics, biocides, non-steroidal anti-inflammatory drugs (NSAIDs), and heavy metals, along with environmental factors such as temperature and precipitation, might enhance the selection of antibiotic-resistant bacteria (ARB) even at low concentrations, inadvertently promoting the proliferation of ARGs.

Since WWTPs can vary in their removal efficiency and influent compositions, it is important to assess their role on influencing the relative abundance of ARGs and pollutant concentrations in discharged effluent.

**Methods:** We collected data between September 2021 and to January 2023 across three distinct wastewater continuums: hospital-based, urban non-touristic, and urban touristic in Guadeloupe, French Caribbean. Using 16S rRNA gene sequencing, mass spectrometry, we investigated changes in the resistome and exposome within untreated wastewater influents and treated effluents from three WWTPs. The multidimensional data was analysed using Random Forest Algorithm, and lasso analysis.

**Results:** The results revealed that the reduction in ARG abundance by the WWTPs was lower than expected. Of the 16 clinically relevant genes and mobile genetic elements examined, the relative abundance of *aph(3")-III*, *blaOXA*, *blaSHV*, *blaTEM*, *ermB*, *intI1*, *qnrS* and *tetM* was reduced by 59.8% to 89.9% across the three WWTPs. An increasing trend in the relative abundance of the *mcr-1* gene was observed, showing a 9.52-fold increase in the treated effluent in the hospital continuum. Among the nine antibiotics tested, effluent concentrations of ciprofloxacin and trimethoprim were reduced by 64.4% to 78.8% in both touristic and non-touristic continuums, while no other antibiotics or biocides among the eight tested were effectively reduced. Regarding heavy metals, significant reductions were

observed only in the non-touristic continuum, with cadmium (Cd), copper (Cu), and mercury (Hg) concentrations reduced by 32.5%, 21.1%, and 36.2%, respectively.

Biocides, the antibiotic erythromycin, some selected heavy metals (As, Cd, Cu, Cr, and Hg) and water temperature were associated with the relative abundance of clinically relevant ARGs.

**Conclusions:** Antibiotic resistance genes were globally reduced by WWTPs over the study. Our results provide insights into the associations between anthropogenic activities, environmental factors and the dynamics of antibiotic resistance genes in effluents in Guadeloupe.

## P-106

# Pollution in an Aquatic Highway: Spread of Antimicrobial resistance along the Mekong River from Cambodia to Vietnam

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**Background and objectives:** The Mekong River, the longest river in Southeast Asia, flows through a catchment area inhabited by over 100 million people, where antibiotics are used and released without proper regulation. The study aims to explore the AMR patterns and their dynamics along the Mekong River in Cambodia and Vietnam by characterizing the antimicrobial resistance genes (ARGs) and bacterial population distribution.

**Methods:** 9 water samples from Cambodia and 18 from Vietnam were collected along the Mekong River during the rainy season. DNA from all samples was extracted and used for shotgun metagenomics sequencing on Illumina HiSeq XTen instrument. The generated raw reads were analyzed by ARGs-OAP (v3.2.4) to detect the relative abundance of ARGs, and by Bracken (v2.9) to evaluate the abundance of species from the DNA sequences. Nf-core/mag (v2.5.4) workflow is subsequently used for assembly, binning, and annotation of the metagenomes. Finally, metamobilepicker (v.0.7.2) was used to identify contigs that co-carry ARGs and mobile genetic elements (MGEs).

**Results:** ARGs-OAP analysis showed that the five most abundant resistance types (80% of all identified ARGs) included multidrug resistance type (38.99%) and beta-lactam (12.97%), tetracycline (11.29%), sulfonamide (8.63%), bacitracin (8.12%) resistance types. Among the ARGs associated with resistance to last-resort antibiotics including carbapenems, polymyxins, vancomycin, and tigecycline, blaOXA was the most abundant gene family. Many of the detected ARGs, particularly sul, bla, florR, tet, and aph, were predominantly carried on plasmid contigs, suggesting potential horizontal gene transfers (HGTs) that could facilitate the AMR dissemination. Furthermore, higher ARG abundances were observed in samples from Can Tho and the sampling sites from downstream in Vietnam. ARG abundances were clustered distinctly by countries, with samples from Vietnam exhibiting higher relative abundances compared to those from Cambodia. Bracken analysis showed that Pseudomonadota, Actinomycetota, Cyanobacteriota, Bacillota, and Bacteroidota were the five most abundant bacterial phyla detected. Remarkably, the percentage of classified reads in samples from Cambodia was approximately 6%, lower than that from Vietnam, which ranged from 16.2% to 54.7%. Consequently, different bacterial community distribution patterns were observed between the two countries. Notably, the bacteria taxa found in samples from Cambodia consisted of a diverse bacterial phyla, whereas samples from Vietnam were mostly Pseudomonadota.

**Conclusions:** The data shows a high diversity of bacteria species as expected, but also ARGs along the Mekong. Antimicrobial resistance appears more prevalent in Vietnam than in Cambodia, as indicated by a higher relative abundance of ARGs detected downstream of the River. This increased ARG abundance along the Mekong could be linked to bacteria belonging to the Pseudomonadota phylum, since this phylum is more represented in Vietnam. In addition, higher abundances of ARGs were observed in sites near large urban areas such as Can Tho, suggesting the existence of hot spots that need to be further studied. Supplementary analyses through a longitudinal study are thus necessary to validate these results, explain the differential patterns of ARGs and bacterial populations between the two countries and notably, define the spatio-temporal spread mechanisms of AMR in order to identify the factors associated with hotspots.

## P-107

# Genomic characterization of an *Enterobacter asburiae* harbouring a conjugative *bla*IMI-6-plasmid isolated from a public garden in Switzerland

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**Background and objectives:** Carbapenemase-producing *Enterobacterales* (CPE) are a major health-care concern challenging both human and animal medicine. We screened a well-visited public garden where people and animals gather to determine if CPEs are also present in such environments.

**Methods:** Twelve samples were taken with sterile wipes from the soil surface in different areas of approximately 6 m2 and spread onto CHROMID CARBA agar. Antimicrobial susceptibility was determined by MIC measurement and carbapenemase production by Blue Carba test. A complete circular genome was obtained by hybrid assembly (Unicycler) of both Oxford Nanopore and Illumina reads. Sequence typing (ST), antimicrobial resistance gene (ARG) screening, plasmid replicon typing, cgSNP analysis and comparative plasmid analysis were performed using bioinformatic tools. Plasmid transfer to *E. coli* was tested by filter mating.

**Results:** One *Enterobacter asburiae* strain (19YS-C) was isolated from a sample taken around a trash bin. 19YS-C carried ARGs associated with resistance to β-lactams (*bla*ACT-10, *bla*IMI-6) and fosfomycin (*fosA*). The *bla*IMI-6 gene was located on a 163-kb IncFII(Yp) plasmid p19YS-IMI-6, which shared the same plasmid backbone as five other *bla*IMI-6-containing plasmids deposited in GenBank. 19YS-C belonged to ST657 and was most closely related (79 SNPs) to another *E. asburiae* ST657 previously isolated from a pre-washed retailed salad in Switzerland [1], which also harboured a *bla*IMI-6-containing IncFII(Yp) plasmid. Plasmid p19YS-IMI-6 was transferred into *E. coli* at a frequency of 10-7 transconjugants per donor.

**Conclusions:** Screening of a public garden led to the detection of an *E. asburiae* harbouring *bla*IMI-6, which is considered as a minor Ambler class A carbapenemase as IMI are infrequently detected [2]. Detection of an IMI-6-producing strain around a trash bin, which is highly similar to another strain isolated from a retailed salad, suggests that food and food waste are possible dissemination vehicles of CPEs. Thus, they could reach the community and spread further in the human and veterinary settings.

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**P-108**

# Dissemination of Antimicrobial Resistance in Klebsiella spp. from Urban Aquatic Environments: A Multi-Country Genomic Perspective

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**Background and objectives:** Antibiotic resistance, particularly carbapenem-resistant *Klebsiella pneumoniae* (CRKP), poses significant clinical and environmental threats, especially in urban aquatic ecosystems and hospital wastewaters [1], community and clinical environments. Rivers and hospital wastewaters can serve as reservoirs for antibiotic-resistant bacteria, posing a risk for the further spread of resistance genes. This study aims to analyze the epidemiological and genomic features of 192 CRKP isolates in urban aquatic environments and evaluate their public health and environmental impacts.

**Methods:** Water samples were collected from 113 rivers and 3 hospitals in China, Sri Lanka, and Nepal to isolate carbapenem-resistant *Klebsiella* spp. isolates. Antimicrobial susceptibility testing [2], whole-genome sequencing, and bioinformatics analyses were performed to characterize resistance phenotypes, antibiotic resistance genes (ARGs), and evolutionary trends. Big data analysis further elucidated the genomic characteristics of CRKP in global water sources, and *Galleria mellonella* larvae were used to assess virulence. Statistical analysis validated the findings.

**Results:** Standard isolation procedures and phenotypic tests confirmed 192 carbapenem-resistant *Klebsiella* spp. isolates isolated from urban aquatic ecosystems in China (n = 60) and Nepal (n = 132), with CRKP (n = 161) being the predominant species. All CRKP isolates exhibited a multidrug-resistant phenotype, yet significant differences in resistance profiles and associated ARGs were observed between isolates from the two countries. Whole-genome sequencing (WGS) and bioinformatics analyses identified nine distinct carbapenem resistance genes (CRGs) were detected, with *bla*NDM-1 being the most prevalent (57.8%). Correlation analysis revealed a strong association between these CRGs and multiple Inc-type plasmids, suggesting active plasmid-mediated dissemination. Phylogenetic analysis of global waterborne CRKP isolates revealed extensive genetic diversity, highlighting multiple sequence types (STs) and serotypes, including hypervirulent lineages. Global genomic analysis of CRKP from water sources across eight countries identified ten distinct CRGs across 45 serotypes, with KL64 being the most predominant. Notably, carbapenem-resistant hypervirulent Klebsiella pneumoniae was detected in water samples from Nepal, based on *Galleria mellonella* infection model.

**Conclusions:** Our findings highlight significant regional disparities in CRKP prevalence and ARG dissemination across urban aquatic environments, with Nepal showing the highest prevalence, particularly in untreated rivers. China exhibited lower prevalence but distinct resistance gene profiles, while no CRKP was detected in Sri Lanka, underscoring the impact of environmental management and healthcare infrastructure on ARG spread.

**Figure**

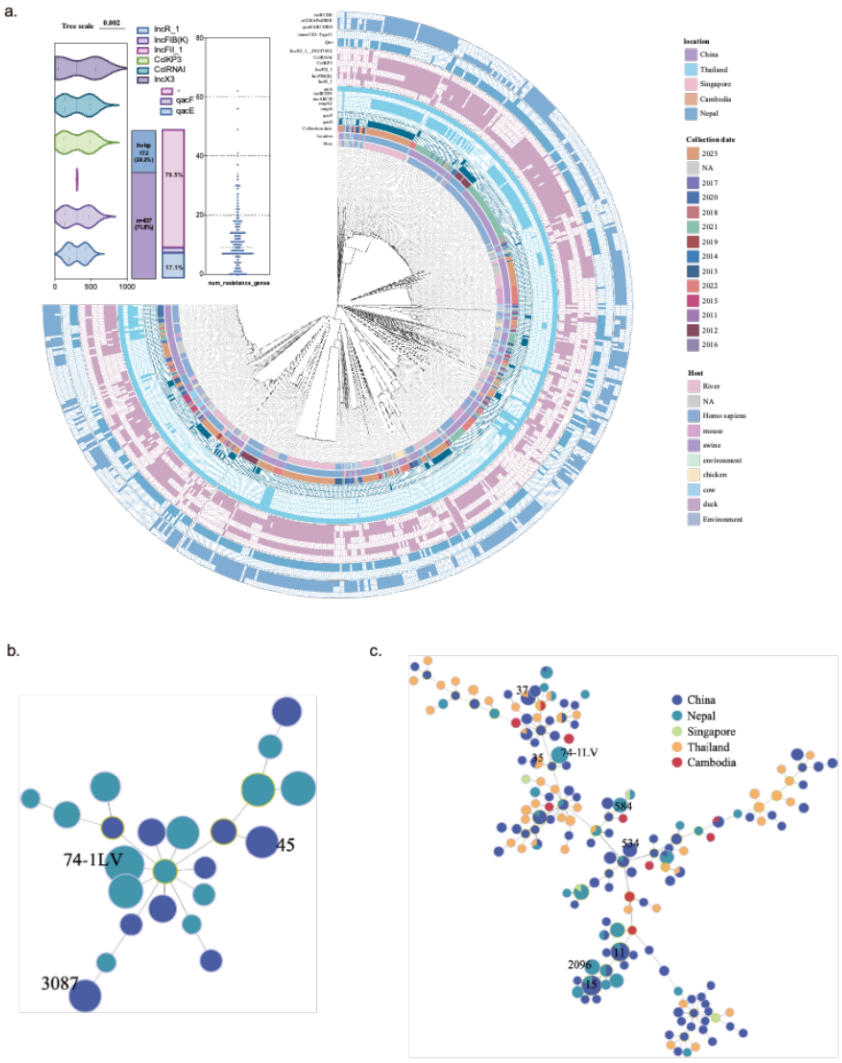


Figure 3. Phylogeny and Minimal spanning tree of CRKP. a. Phylogenetic tree of CRKP isolates from Southeast Asian countries. Source, host, plasmids, ARGs, virulence resistance genes, chlorine disinfection tolerance gene (*qacE*), and heavy metal resistance gene (MRG) were annotated. Branches of strains belonging to our study were labelled with red colors. b. STs distribution of CRKP isolates in this study. c. STs distribution of CRKP isolates in Southeast Asian countries.

**P-109**

# Exploring the impact of agricultural waste treatment on microbial diversity and AMR gene burden

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**Background and objectives:** The increasing global concern over antimicrobial resistance (AMR) highlights the need to understand how agricultural waste treatment processes affect microbial communities and AMR gene prevalence. This pilot study investigated the metagenomic structure of agricultural waste and evaluated the effects of anaerobic digestion (AD) and on-farm slurry lagoon treatments on microbial diversity and AMR gene abundance. The hypothesis was that waste treatment would alter community composition, reducing both AMR gene burden and the abundance of clinically relevant Enterobacteriaceae.

**Methods:** Samples were collected from two timepoints (before and after treatment) at three AD plant sites and two on-farm slurry lagoon sites. DNA was extracted from each sample, followed by short-read Illumina sequencing to a depth of 5 Gb. Reads were host-depleted and processed using fastp, then taxonomically classified with Kraken2. Normalised abundance data (via DESeq2) were used to compute alpha (richness, Shannon, Simpson) and beta diversity metrics, visualised through heatmaps and principal coordinate analyses (PCoA). AMR gene presence and abundance was determined using an in-house custom pipeline (APHA SeqFinder), with differential abundance testing (Wilcoxon rank-sum and Kruskal-Wallis tests with Benjamini-Hochberg correction) applied to identify trends in both microbial and AMR gene profiles.

**Results:** Waste treatment induced distinct shifts in microbial composition between AD plants and farm sites. AD plant samples generally showed a reduction in genera richness and evenness, along with a marked loss of high-abundance taxa (including Enterobacteriaceae such as Escherichia and Enterococcus), whereas farm sites maintained stable diversity. PCoA analyses further confirmed larger microbial shifts in AD plant sites. Trends in AMR gene abundance revealed that AD treatment was associated with decreases in several resistance genes (notably macrolide, aminoglycoside, fusidic acid, and beta-lactam resistance genes), while on-farm slurry lagoon treatment had minimal impact. Overall, the study revealed trends showing differential impact of treatment type on both microbial communities and AMR gene profiles.

**Conclusions:** The study provides tentative evidence that anaerobic digestion and on-farm slurry lagoon treatments differentially alter both microbial diversity and AMR gene burden in agricultural waste. These preliminary findings suggest that agricultural waste treatment can decrease the microbial diversity and AMR burden to mitigate environmental AMR risks, but further follow-up studies are required in future to confirm these observations and optimise treatment protocols.

**P-110**

# Effect of Biocides on the Transfer of Antibiotic Resistance Genes

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**Background and objectives:** Biocides are widely used in farm and livestock environments for routine cleaning and disinfection to prevent and control disease. However, evidence exists that certain biocides at sub-inhibitory levels – conditions that may be encountered in natural settings - can

enhance bacterial conjugation rates. For instance, quaternary ammonium salts (QACs), which are used on farms, have been shown to promote conjugation, thus contributing to bacterial resistance to multiple antibiotics [1]. However, the effects of many other authorised biocides on horizontal gene transfer remain largely unknown. This study aimed to investigate the impact of four commonly used farm disinfectants containing different active ingredients (chlorocresol, glutaraldehyde and formaldehyde, potassium peroxymonosulphate and iodine) on conjugation rates.

**Methods:** Conjugation experiments were conducted using a rifampicin-resistant *Salmonella enterica* recipient strain and three *Escherichia coli* donor strains harbouring different plasmids: one carrying amikacin (*rmtB*) resistance on an IncFII plasmid, a second with cefotaxime (*CTX-M-14*) and ciprofloxacin (*qnrS1*) resistance on an IncI plasmid, and a third with cefotaxime (*CTX-M-1*) resistance on an IncI plasmid. Conjugations were performed in the presence of disinfectants at sub-minimum bactericidal concentrations (sub-MBC) and absence of disinfectants (controls). Growth and conjugation rates were compared to controls to assess the effect of each disinfectant on emergence of transconjugants. Whole-genome sequencing (WGS) was performed to confirm plasmid transfer and characterise transconjugants.

**Results:** Disinfectants containing chlorocresol and potassium peroxymonosulphate inhibited bacterial conjugations without affecting the viability of recipient and donor strains. Glutaraldehyde- and formaldehyde-based disinfectant had a suppressive effect on conjugations by significantly delaying transconjugant emergence and maintaining lower conjugation rates compared to controls. In contrast, iodine-based disinfectant resulted in conjugation rates similar to or, higher than controls, suggesting a potential promoting effect on horizontal gene transfer.

**Conclusions:** Biocides are routinely used to control microbial growth. Our findings indicate that at sub-inhibitory levels, some biocides may suppress while others enhance horizontal gene transfer. Since horizontal transfer plays a key role in the spread of mobile elements harbouring resistance genes, these findings raise concerns about the potential implications of biocide in antimicrobial resistance dissemination on farms.

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## P-111

# Metagenomic analysis of the airborne antibiotic resistome and mobilome in Belgrade metropolitan area during fall season

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**Background and objectives:** Antimicrobial resistance (AMR) has been identified as one of the top three threats to public health in the 21st century. The role of the environment in the spread of antimicrobial resistance is increasingly recognized. The airborne route for the transmission of antibiotic resistance genes (ARGs) is particularly important as bioaerosols carrying ARGs can travel long distances and remain in the atmosphere for long periods of time. In addition, mobile genetic elements (MGEs) can facilitate the spread of ARGs in the environment. Considering that Serbia is a country struggling with overuse and misuse of antibiotics, a high percentage of multidrug-resistant bacterial isolates and poor air quality, the Serbian capital Belgrade was recognized as an interesting research model for studying the airborne transmission of AMR.

**Methods:** Outdoor air was sampled with hydrophobic polypropylene membrane filters (air volume of 90 m3) at seven differently categorized locations: industrial (Veliki Crljeni and Barajevo), traffic (Borča, Despota Stefana, Leštane, Banovo Brdo) and background (Zeleno brdo) during the fall season 2024.

The DNA samples from the air were subjected to shotgun metagenome sequencing (Illumina Novaseq X plus) and bioinformatic analysis (Novogene, UK). ARGs and MGEs were annotated using CARD, Integrall, Isfinder and Plasmid databases, respectively.

**Results:** Metagenomic analysis revealed that the air sampled in Barajevo had the highest relative abundance of ARGs, with antibiotic transformation/deactivation being the predominant resistance mechanism, while the lowest value was observed in the Borča sample. In addition, the ARG profile varied between samples, although Zeleno Brdo/Borča and Banovo Brdo/Veliki Crljeni showed similar ARG patterns. The most abundant ARG in almost all samples was *bla*TEM. Among the MGEs, over 20 insertion sequences were revealed when analysed with ISfinder, with 5 out of 7 sites showing similar patterns, while Barajevo and Despota Stefana fell out of the group. The same observation was made when the plasmid profile of the samples was compared using the Plasmid database. In contrast, integron-related sequences differ significantly among the samples.

**Conclusions:** This study revealed that the air in Belgrade could be a potential dissemination route for antimicrobial resistance.

## P-112

# Surface water as a reservoir for klebsiellae? Diversity and potential impact of isolates from natural and human-associated sources on Haiti

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**Background and objectives:** Klebsiellae are nosocomial pathogens increasingly notified by public health services. Due to their high adaptability and their ability to acquire foreign DNAs, human infections with MDR-isolates are challenging to treat. In addition, environmental klebsiellae are also reliable indicators for the dynamics in resistance acquisition in ecosystems caused by pollutions forcing the adaption of these bacteria. As comprehensive information from different ecosystems is lacking, klebsiellae from surface/wastewater were investigated in Haiti.

**Methods:** Here we report on a broad collection of *Klebsiella* sp. isolates recovered from 12 stations during an environmental *Vibrio* survey in 2021. Isolates were subjected to pulsed-field gel electrophoresis for genome profiling (XbaI-PFGE), S1-PFGE for plasmid profiling and whole-genome sequencing (WGS). Bioinformatic analysis was used to determine genome typing and to detect and localize antimicrobial resistances. Plasmid-associated resistances were subjected to *in-vitro* filter mating studies to assess their transmissibility.

**Results:** PFGE revealed a wide variety of different genomic XbaI patterns across the different sampling points. Phenotypically, only some isolates show resistance to the antimicrobials tested, which was found to be in good agreement with the resistance genes determined by WGS. In addition, some of the isolates also exhibited strong hypermucoviscosity and transmissible plasmid-associated AMR. The *in-silico* analysis of klebsiellae genomes provides a detailed insight into their genetics and potential impact on human health.

**Conclusions:** Analysis of environmental klebsiellae provides information on sources of antimicrobial resistance acquisition, hotspots for bacterial evolution, and the general occurrence of clinically relevant lineages that may affect the health of the local human population through colonization of susceptible individuals.

**P-113**

## Diversity and Distribution of Metal and Biocide Resistance Genes in Airborne Metagenomes Across Urban Locations in Belgrade, Serbia

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**Background and objectives:** Air as a medium for the transmission of resistance genes remains an understudied environment. While antibiotic resistance is currently the most discussed topic, investigating resistance to other pollutants, such as metals and biocides, can provide deeper insights into the reservoirs and dissemination of resistance genes, as well as their connection to environmental pollution. Additionally, mobile genetic elements often carry not only antibiotic resistance genes but also genes conferring resistance to metals (MRG) and biocides (BRG). Here, we present an analysis of the presence and diversity of MRG and BRG in airborne metagenomes collected from seven locations within the city of Belgrade, Serbia, during the summer of 2024.

**Methods:** Air samples were collected using hydrophobic polypropylene membrane filters during the summer period at seven locations: Barajevo (BA), Borča (BO), Despota Stefana (DS), Leštane (LE), Veliki Crljeni (VC), Zeleno Brdo (ZB), and Zemun (ZU). Total DNA was isolated using the classical phenol-chloroform method. The isolated DNA was sequenced on the Illumina NovaSeq X Plus platform, and bioinformatic analysis was conducted at Novogene, UK. Subsequent *in silico* analysis was performed using the AMR++ v3.0 pipeline with the MEGARes database.

**Results:** The seven locations (BA, BO, DS, LE, VC, ZB, ZU) exhibited relative abundances of MRG at 14.34%, 7.62%, 15.27%, 10.25%, 8.31%, 4.73%, and 24.49%, respectively. For BRG, the relative abundances were 4.92%, 2.21%, 5.32%, 0.68%, 1.07%, 1.81%, and 9.41%, respectively. Variations in the abundance of specific genes were observed; for instance, the iron resistance gene (*acn*) was detected at locations DS and VC but was absent at other sites. Additionally, genes conferring resistance to tellurium (*klab* and *klac*) were found in high abundance (62%) at location LE

**Conclusions:** This study demonstrates the presence of MRG, and BRG in airborne environments, with significant spatial variations across different locations in Belgrade. The detection of specific genes, such as those for iron and tellurium resistance, at higher abundances in certain areas suggests localized sources or environmental factors influencing their distribution. These findings underscore the potential role of air as a reservoir and transmission route for resistance genes and highlight the need for further research into the dynamics of airborne environments in the spread of antimicrobial resistance.

**P-114**

## Monitoring and reporting on antimicrobial resistance (AMR) in surface waters

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**Background and objectives:** The occurrence and proliferation of antibiotic-resistant bacteria (ARB) and the transmission of resistance through gene transfer can be of great importance to human health. However, the role of the environment, particularly the introduction of ARBs and antibiotic resistance genes (ARG) into surface waters, is not yet fully understood within the One Health framework, necessitating further research in this area. Therefore, it is important to evaluate technical process

chains regarding ARG and ARB retention and develop a well-founded data basis for the occurrence of AMR in comparison to the general bacteria load in the different seasons of the year, investigating different sampling sites in Germany. The species which are relevant in the monitoring are clinically representatives of ESBL-producing Enterobacteriaceae and VRE-producing enterococci [1].

**Methods:** The focus was on selecting a limited number of common and relevant bacteria and ARGs as well as appropriate, standardized, and valid cultural and molecular biological methods for their detection.

One major objective was the validation of cultural detection methods for a better comparison of ARB detection in aquatic environmental samples. Both clinically relevant representatives of ESBL-producing Enterobacteriaceae (*Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Enterobacter* spp., *Escherichia coli*, *Citrobacter* spp., *Serratia* spp.) and VRE-producing enterococci on CHROMagar™ ESBL and CHROMagar™ VRE media were examined. In order to use the methodology beyond research projects, the requirements of DIN EN ISO/IEC 17025 (2018) [2] are necessary, which is why the performance data is collected in accordance with DIN EN ISO 13843 (2018) [3]. For the detection of *E. coli*, tests were also carried out with TBX agar containing cefotaxime in order to assess the practicability of different media.

For the validation of molecular biological detection methods, a base vector (pBE1) with a polylinker for cloning of resistance genes via Golden Gate assembly was generated. qPCR enables the quantification of total bacteria abundance (16S rDNA gene) and specific resistant genes (bla-KPC, bla-TEM, bla-CTX, ermB, vanA, aadA1, intI).

**Results:** The current studies are still in progress to achieve a comprehensive data basis on AMR in surface waters. We successfully developed a harmonized methodology encompassing monitoring objectives, sampling locations (surface water, rivers downstream of waste-water-treatment points (WWTP)), target indicators (*E. coli*, ESBL-*E. coli*, coliforms bacteria, ESBL-coliforms bacteria, enterococci, VRE-enterococci and eight core ARGs with optional additions), and analytical methods (culture-based and qPCR).

**Conclusions:** In conclusion, monitoring AMR in surface waters using reliable and robust methods is of great importance to establish environmental quality criteria for AMR abundance. Furthermore, the present studies show valuable insights into the challenges and complexities of environmental AMR surveillance.

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**P-115**

# *Aeromonas* spp. a "one health" indicator of antimicrobial resistance dissemination?

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**Background and objectives:** To monitor antimicrobial resistance (AMR) dissemination, a one health approach is needed, including human, animal and environment. Should we use the same indicator for the three compartments? *E. coli* is used as indicator of AMR dissemination for human and terrestrial animals. *Aeromonas* is an autochthonous bacterium of aquatic environment, proposed as an indicator of AMR in aquatic environment and aquaculture. The aim of this study was to investigate whether *Aeromonas* could be an AMR indicator in terrestrial food producing animals.

**Methods:** Fresh fecal samples were collected in four types of food-producing animals: pig, poultry, turkey and rabbit. All farms were located in North-Western part of France. At their arrival in the lab, samples were stored with and without glycerol at -70°C until processing. Detection of *Aeromonas* was done by cultivation method using Glutamate Starch Pseudomonas agar and/or by PCR[1]. *E. coli* detection was done by PCR[2]. Total microflora was estimated by culture method on Tryptone Soy Agar and PCR[3]. DNA extract was performed using the le kit NucleoMag Tissue ®Macherey Nagel. PCR was done on pure and diluted DNA extracts (1/10 and 1/100).

**Results:** The 205 fecal samples were collected between 2019 and 2021. From four pig farms, 160 samples were taken on sows, piglets and fattening pigs. From seven turkey farms 24 samples were taken on male and female broilers. The seven rabbit samples were collected from three farms (one was sampled twice). The 14 poultry samples were collected in two farms from two and seven batches respectively.

Culture detection was performed for a subset of five fecal samples (two pigs, one rabbit, one turkey and one poultry). None *Aeromonas* was detected by culture in these five samples at the opposite, *E. coli* was detected in all samples.

By PCR all the 205 samples were negative whatever the dilution of the DNA extract tested for *Aeromonas*. For *E. coli*, 80% of the non diluted samples and 100% of the diluted extracts were positive. Total microflora was detected in all samples by culture and by PCR.

Detection of *E. coli* and total microflora either by culture or PCR prove the quality of the DNA extracts and the storage of the samples.

Non detection of *Aeromonas* could be linked to a very low level of abundances and/or a weak sensitivity of the methods. A low frequency of *Aeromonas* in pig and cow was also reported by Jones et al 2024 where *Aeromonas* was identified in only 59 of 801 (7.4%) cow/pig samples (based on the proportions of reads assigned to the genus *Aeromonas* in 16S rRNA gene sequence).

**Conclusions:** Based on these results, *Aeromonas* seems not to be a suitable indicator for AMR dissemination in terrestrial animal due to the lack of systematic detection with conventional methods.

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**P-116**

# Biodiversity-dependent invasiveness of naive river epilithic biofilms by anthropogenic antibiotic resistance

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**Background and objectives:** With more than 1 million deaths attributed each year, antibiotic resistance has become a major societal issue. The emergence and dissemination of antibiotic resistance in bacteria rests on two pillars, the enrichment of resistant variants upon selection and the contagion of the resistant bacteria and their resistance genes (ARGs) within and across the human, animal and environmental spheres [1]. Although poorly described, this contagion process necessarily implies the persistence of invading resistant bacteria from one microbiome to another. The aim of this work was to characterize the extent of antibiotic resistance invasions in naïve river biofilms and its relationship to the level of biodiversity of these resident bacterial communities.

**Methods:** In this study, we carefully selected a series of watershed head rivers in the Vosges Mountains (northeastern France) with a clear pristine-like upstream part and well identified prim-exposure to modest anthropic activities. Microbial community DNAs were obtained from river epilithic biofilms do determine (i) their profiles of ARGs and mobile genetic elements (EGMs) by high-throughput qPCR (HT-qPCR), and (ii) their community structures by 16S rRNA gene metabarcoding.

**Results:** ARGs/MGEs profiling by HT-qPCR along river continuums showed that one third of the markers were already widespread, while another third massively invaded the river biofilm communities at prim-exposure to anthropic activities, with the concomitant entry of fecal pollution. The analysis of the corresponding biofilm community revealed that the extent of the invasion process was inversely correlated with the level of biodiversity but positively correlated with the magnitude of propagule pressure.

**Conclusions:** Even low anthropic activity elicits massive river invasions by antibiotic resistance, with a magnitude that depends on the level of biodiversity of the resident biofilm communities.

**P-117**

# Temporal and spatial dynamics of the invasion of river biofilms communities by antibiotic resistance

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**Background and objectives:** The emergence and dissemination of antibiotic resistance lies on two pillars, the selection of resistant variants during therapy and the subsequent spread of resistant bacteria (ARB) and their resistance genes (ARGs)[1]. As both recipient of anthropic pollution and sources of exposure, river aquatic environments act as a transmission belt for the spread of ARB/ARG. However, understanding the invasion of ARB/ARGs in rivers remains complex considering the number of existing ARG determinants and the multiplicity of pollution sources. In this work we explored the invasion dynamics of ARB/ARGs in river biofilms from remote streams with well-identified anthropogenic pollution inputs. The reversibility of the invasion phenomenon and the role of the resident community were also investigated.

**Methods:** Remote rivers from the Vosges Mountains (Northeastern France) were selected for lack of anthropic activity in their watershed heads. Epilithic biofilms (resident community) were sampled along of the river continuums and the sites of prim-exposure to ARB/ARGs were identified by high-throughput qPCR for 45 ARGs. Immerged artificial biofilm supports were used to determine the dynamics of invasion processes and its reversibility when biofilms were moved upstream of the pollution source. The structure of biofilm communities was characterized by 16S rRNA gene metabarcoding.

**Results:** The analysis of ARG composition in biofilms collected along the continuum of selected rivers allows identifying the site of prim-exposure to anthropogenic pollution responsible for ARB/ARG invasion. Using artificial supports positioned upstream and downstream the pollution input, we showed that the biofilm community composition differed by less than 20% over a 5-week period and between sites. When biofilms formed upstream of the pollution source are transferred downstream and vice versa, we demonstrated that upstream biofilm communities are more dynamics than the downstream ones. However, both biofilm communities are equally dynamics in terms of ARG invasion and ARG depuration for biofilm re-positioned downstream and upstream the pollution source respectively.

**Conclusions:** Depuration of ARB/ARGs from natural biofilms can occur relatively fast when the anthropogenic pressure cease, without any massive alteration of the microbial community structure.

**P-118**

# Dissemination of antibiotic resistance in a constructed wetland

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**Background and objectives:** The use of constructed wetlands (CWs), a nature-based solution, to improve the physical and chemical quality of Wastewater Treatment Plant (WWTP) effluents before they are released to the environment is gradually increasing. However, WWTPs are not designed to remove antibiotic resistant bacteria, antibiotic resistance genes (ARGs) or Mobile Genetic Elements (MGEs) and their fate while flowing through CW remains poorly understood. Our project aims to study antibiotic resistance and its dissemination in a large pilot CW (6 ha) consisting of three parallel ponds planted with different types of vegetation.

**Methods:** The influent and effluent from the WWTP and the CW and water from a nearby lake (protected area) are sampled monthly. Feces from wildlife (mainly swans) living on the CW or on the nearby lake are also sampled. Total DNA from feces and DNA from bacteria and phages collected separately from water samples are extracted. Then, their content in 3 ARGs (*aacA4*, *aadA*, *aadB*) and 3 MGEs (class 1 integrons, IncF and IncP1 plasmids) are determined by quantitative PCR. Total coliforms are enumerated on m-ColiBlue24 agar and third-generation cephalosporin- and carbapenem-resistant Enterobacterales are isolated on chromogenic selective media. The isolates are phenotypically characterized (antibiograms) and the major clade to which they belong is determined. 16S metabarcoding is performed once per trimester on water sample DNAs.

**Results:** The relative abundances of ARG/MGE (copies per 16S rDNA) vary slightly over time in the WWTP influent but are mainly stable in the WWTP effluent and therefore stable in the CW influent. The abundances of ARGs/MGEs in the CW effluent are more variable from one campaign to another. Although the abundances of ARGs/MGEs in the CW influent and effluent are similar, the composition of the bacterial communities changes very significantly during the course of the water in the CW. Water samples and feces from the nearby lake are less loaded with ARG/MGE and resistant Enterobacterales than those from the WWTP or the CW.

**Conclusions:** Water treatment efficiency in the CW shows a seasonal effect. The bacterial community changes in the CW while the abundances of ARGs/MGEs remain stable suggest community coalescence and/or horizontal gene transfer. These hypotheses are currently being investigated. The high load of resistant Enterobacterales in the feces of wildlife living in the CW strongly suggests a contamination of these animals by anthropogenic bacteria, ARGs and MGEs, making the CW a hotspot of antibiotic resistance dissemination in the ecosystem and beyond. Sampling campaigns will continue to confirm these findings. Whole genome sequencing of resistant Enterobacterales is planned. To extend our monitoring of antibiotic resistance and our understanding of its fate in the CW, high-throughput quantitative PCRs will be performed on a large panel of ARGs/MGEs.

## P-119

# Antimicrobial susceptibility of *Pseudomonas aeruginosa* collected in a river impacted by fishfarming and humane wastewaters

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**Background and objectives:** *Pseudomonas aeruginosa* is a ubiquitous bacterium present in different types of water including wastewater and surface water. This bacterium is also a "member" of Eskape group" which includes the five most important nosocomial human pathogens. Indeed, it has been classified as one of the top three priority pathogen for which new antibiotics are urgently needed [1]

Aquatic environment is considered as a hot spot of antimicrobial dissemination and farming activities are supposed to favor selection of resistant bacteria.

The aim of this study was to characterize the antimicrobial susceptibility of *P. aeruginosa* collected a long a French river during 16 months.

**Methods:** Water samples were collected every two weeks, upstream and downstream two fishfarms one located at the source (F1) and the other (F2) at the month of the river. *P. aeruginosa* was isolated using Pseudomonas aeruginosa agar (Biorad) and up to five colonies by sample were stored. Confirmation of presumptive identification was done by PCR [1]. Antimicrobial susceptibility test was performed by agar diffusion for 20 antibiotics belonging to five classes: Betalactamins (11), Fluoroquinolones (3), Fosfomycin (1), Aminoglycosides (4) and sulfonamide (1) (according to Eucast guidelines. Epidemiological cut off values (Ecoff) defined by Eucast or based on the data of this study, was choosen as interpretative criteria.

**Results:** 252 isolates were identified as *Pseudomonas aeruginosa* (confirmation rate of 39.1%): 59 upstream and 63 downstream F1 and 77 and 53 upstream and downstream the F2. Provisional Ecoff values were determined for seven antibiotics: aztreonam, fosfomycin, cefsulodine, cefepime, piperacillin, sulfonamide, ticarcillin-clavulanic acid.

Only two isolates (0.8%) were non wild type to aminoglycosides (1 to gentamicin and 1 to tobramycin), 58 isolates (23.0%) were non wild type to fluoroquinolones (24 to ciprofloxacin and levofloxacin and 30 only to levofloxacin), (64%) were not wild type to Fosfomycin. Four isolates were non wild type to ticarcilin but one only one was not wild to clavulanate acid-ticarcillin. Only one isolate was non wild type to meropenem

No difference was observed between the proportion of non wild type isolates between upstream and downstream of each fishfarm whatever the antibiotics.

A decrease of the proportion of isolates non wild type to fluoroquinolones was observed from the source (36.5%) to the month (9.2%) of the river.

**Conclusions:** The use of Ecoff allowed monitoring the dissemination of AMR independently to the fate of antibiotic therapy. Our set of data will be submit to Eucast website.

Based on these results, the impact of fishfarming on the antimicrobial susceptibility of *Pseudomonas aeruginosa* seems to be very limited. Investigation of the antimicrobial susceptibility of *Pseudomonas aeruginosa* isolates collected in the output of wastewater treatment plant in this watershed are under investigation The contamination by non wild type isolates at the source of the river has to be investigated

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## *t05 - Novel approaches to combat antimicrobial resistance*

### P-120

# Control technologies to prevent spread of environmental antibiotic resistance in the production and use of veterinary antibiotics

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**Background and objectives:** Fermentation production and use of veterinary antibiotics were the hotspots of antimicrobial resistance (AMR) dissemination in the environment. Here, we aimed to uncover the contamination of antibiotics and development of AMR during the treatment of antibiotic production wastewater and livestock wastewater, and develop novel technologies to block the release of these pollutants to the environment.

**Methods:** Full-scale investigation of AMR characteristics in treatment facilities of antibiotic production wastewater and livestock wastewater was conducted, and selective removal techniques of antibiotics and clinically relevant antibiotic resistance genes (ARGs) were developed.

**Results:** The widely used biological systems, usually a combination of anaerobic digestion and activated sludge process, are vulnerable to the presence of extremely high concentrations of antibiotics in antibiotic production wastewater. Heavy multidrug resistance would be developed during wastewater treatment due to horizontal gene transfer of ARGs among bacterial community mainly through the enrichment of plasmids harboring multidrug resistance regions. Based on the above findings, pre-treatment of production wastewater to remove antimicrobials is the best way to control the development of AMR during the biological wastewater treatment. Enlightening by the easy-to-hydrolyze property of most of antibiotics, we have developed a novel pretreatment technology based on enhanced hydrolysis by using homogeneous or heterogeneous solid acid/base catalysts for targeted elimination of antibiotic potencies from wastewater. Then this technology has been successfully applied to the treatment of oxytetracycline manufacturing wastewater in two sites in Hebei Province (800 m3/d and 1,000 m3/d, respectively). The abundance of ARGs in the biological treatment units could be reduced by more than 83%, and the challenge on biological inhibition was also solved. In addition, hydrothermal treatment based on enhanced hydrolysis was also applied in full-scale plants in China for recycling waste erythromycin and cephalosporin fermentation residues. Livestock wastewater was another major discharge source of ARGs in the environment. About 300 *bla*CTX-M-carrying *E. coli* strains and *optrA*-carrying *Enterococcus* strains were isolated from mesophilic anaerobic digestion effluents treating manure wastewater. Mobile *tet*(X4) was also found as the dominant *tet*(X) variant, and persisted within anaerobic digestion treatments. Hyperthermophilic-mesophilic two-stage anaerobic digestion could reduce the abundance of *bla*CTX-M and *optrA*, and inactivate the fecal bacteria effectively, indicating effective management of operating temperature in

anaerobic digestion should be implemented to prevent the discharge of the clinically relevant ARGs from the animal farms.

**Conclusions:** Antibiotic production wastewater pretreatment technology based on enhanced hydrolysis and livestock wastewater treatment technology based on hyperthermophilic-mesophilic two-stage anaerobic digestion were developed to prevent the spread of environmental antibiotic resistance.

## P-121

# Comparison of disk and media lots for *Escherichia coli* ATCC® 25922 and oxytetracycline 30µg disks

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**Background and objectives:** Antimicrobial resistance renders antimicrobial susceptibility testing increasingly important to predict the outcome of antimicrobial therapy. Therefore, a validation with quality control (QC) strains and approved QC ranges is essential. One aim of this project (funded by the Federal Office of Consumer Protection and Food Safety (BVL)) was to compare oxytetracycline 30µg disk and media lots for *Escherichia coli* ATCC® 25922 as a pre-requisite to establish QC ranges.

**Methods:** Eight participating laboratories tested two lots of oxytetracycline 30µg disks (ThermoFisher Scientific, Henningsdorf, Germany; Mast Diagnostica GmbH, Rheinfeld, Germany) ten times on three lots of Mueller-Hinton agar (ThermoFisher Scientific, Becton Dickinson GmbH, Heidelberg, Germany, Mast Diagnostica GmbH) by agar disk diffusion according to CLSI and EUCAST in an interlaboratory trial. As quality control, each laboratory tested one lot tetracycline 30µg disks (ThermoFisher Scientific) on one medium lot in parallel. The data was analyzed by using the RangeFinder software [1].

**Results:** In total 480 oxytetracycline and 80 tetracycline data points were evaluated, with all tetracycline results being within the QC range. Oxytetracycline zone diameters ranged from 17 mm to 30 mm. Disk lots revealed mean values of 23.85 mm (standard deviation (SD): 2.48 mm) and 22.16 mm (SD: 2.39 mm). Media lots A, B, and C revealed mean values of 21.47 mm (SD: 1.75 mm), 25.76 mm (SD: 1.54 mm) and 21.78 mm (SD: 1.75mm), respectively, i.e. media lot B displayed a mean value about 4 mm larger than lots A and C. On a laboratory level, the mean values ranged from 22.35 mm to 24.00 mm. For all media lots a range of 17 mm to 29 mm was calculated (99.8% values included) while media lots A and C resulted in 18 mm to 26 mm (98.1% values included).

**Conclusions:** These results show that the agar disk diffusion results can distinctly differ, when using different media/disk lots. Therefore, using disks and media producing similar results is essential for establishing QC ranges. In case a quality control is out of range, the use of an alternative lot of media or disks might be one option to check.

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## P-122

# Bone cement with antibacterial substances against pathogens involved in periprosthetic joint infections

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**Background and objectives:** Surgical replacement of destructive joints with prostheses is a common procedure in both human and veterinary medicine. Bacterial infections at the implant site lead to septic loosening, which often causes implant failure. To combat these infections, antibiotic-laden bone cement is commercially available and generally used to fasten joint implants. However, increasing occurrence of multi-resistant bacteria and failure to manage periprosthetic joint infections make it necessary to identify new antibacterial substances for this purpose. N-acetylcysteine (NAC) and methylglyoxal (MGO) have been chosen as candidate substances due to their potential antibacterial activity. The aim of this project is to evaluate the suitability of the candidate substances in conjunction with bone cement in a clinical context.

**Methods:** Determination of minimal inhibitory concentrations (MICs) was performed with three clinical isolates of *Staphylococcus (S.) pseudintermedius* in broth microdilutions. Cytotoxic concentrations of the substances were measured with viability and proliferation assays after treatment of human osteosarcoma (HOS) cells. Biocompatibility of bone cement supplemented with NAC and MGO was tested by treatment of HOS cells with cell culture medium incubated with bone cement platelets and by seeding HOS cells on substance-containing bone cement platelets. Enzyme-linked immunosorbent assays were used to measure the release of proinflammatory cytokine Interleukin-6 (IL-6). Activation of MAP kinase p38 was analysed by western blotting. In infection experiments, the influence of NAC and MGO below the MIC on adherence and internalisation of *S. pseudintermedius* to HOS cells was analysed.

**Results:** The half-maximal inhibitory concentration (IC50) of NAC for viability of human osteosarcoma cells (HOS) was 3.6 mg/mL and the IC50 of MGO was 0.17 mg/mL. For three clinical isolates of *S. pseudintermedius*, MICs were 2.5 mg/mL for NAC and 0.15 mg/mL for MGO. Supernatants of bone cement platelets supplemented with 50 mg and 100 mg NAC reduced cell viability and proliferation compared to supernatants of bone cement without additive, but release of IL-6 could not be detected. Western blots indicated that p38 could be activated following treatment with NAC. Supernatants of bone cement supplemented with 25 mg MGO reduced proliferation compared to supernatants of bone cement without additive. Substance supplementation led to reduced cell growth on bone cement. NAC did not prevent internalisation and adherence of *S. pseudintermedius* to HOS cells in co-culture experiments, while MGO reduced internalisation and adherence compared to control.

**Conclusions:** Taken together, this study aims to determine effective concentrations of new antibacterial substances in bone cement, while ensuring a high cell compatibility.

**P-123**

# Bacteriophage Therapy for Canine Otitis Externa: In Vitro and Ex Vivo Evaluation

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**Background and objectives:** Otitis externa is a common disease in dogs, which can be caused by *Pseudomonas aeruginosa.* The treatment of *Pseudomonas*-induced otitis is often a big challenge due to the ability to form biofilms and the high intrinsic resistance to the commonly used antibiotics. The bacteriophage (phage) therapy presents a promising alternative treatment option. In this study the bacteriophages were characterized and their efficacy was investigated using *ex vivo* and *in vitro* models of canine skin.

**Methods:** The phages were characterized by whole genome sequencing, host range determination, crystal violet biofilm degradation assay and physical stability testing The phage suspensions were purified by filtration, density gradient centrifugation and dialysis to minimize endotoxins, which are potentially harmful for therapeutic use. The antimicrobial effect of two phages (JG003 and PTLAW1) was tested as a topical application in an artificial infection model on canine skin with Franz-type diffusion cells. The bacterial load was assessed using CFU quantification and immunohistochemistry. To test the effect of the phage therapy on infection-associated mediators, we established an artificial infection on a canine epidermal equivalent, where RT-qPCR and ELISA were used as additional readouts.

**Results:** The characterization of the phage revealed that they have a broad host range, biofilm degradation potential and no known resistance or virulence factors were found. *In vitro* (n=3) and *ex vivo* (n=3) models demonstrated a statistically significant 4-log reduction in bacterial burden in the phage treated samples compared to PBS-treated control. The infection of the epidermal model with *P. aeruginosa* resulted in increased expression of the inflammation-associated mediators (SAA1 and CXCL8), whereas the bacteriophages were able to significantly reduce this expression. This finding was also confirmed via the canine CXCL8 ELISA. Indirect immunofluorescence staining with *P. aeruginosa*-specific antibodies showed a reduced fluorescent signal in the phage-treated samples, further supporting the antimicrobial efficacy of the therapy.

**Conclusions:** The results demonstrate that bacteriophages effectively control *Pseudomonas* infections *in vitro* and *ex vivo*. Their stability, broad host range towards clinical isolates, the ability to degrade biofilms highlight their potential for therapeutic use. Further clinical studies are needed to confirm their efficacy in veterinary patients.

## P-124

# Zidebactam restores cefiderocol sensitivity in resistant bacteria

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**Background and objectives:** Cefiderocol (FDC) is the first approved siderophore cephalosporin with broad-spectrum antimicrobial activity against multidrug-resistant (MDR) gram-negative bacteria [1]. Unfortunately, the emergence of cefiderocol-resistant gram-negative strains limit its clinical application [2]. Here, we explored a synergistic bacteriostatic effect of the FDA-approved drug, Zidebactam (Zid), in combination with cefiderocol.

**Methods:** This research comprehensively investigated the synergistic effect of Zidebactam (Zid) combined with Cefiderocol (FDC) against resistant bacteria. Experimental methodologies, including the checkerboard assay [3], time-kill assay, scanning electron microscopy [4], and cell infection model, were employed to evaluate antibacterial activities, bacterial morphological changes, and intracellular bactericidal efficacy. Additionally, transcriptomic analysis was performed to explore the underlying mechanisms of the Zid-FDC combination.

**Results:** Zid exhibits a synergistic effect with cefiderocol in eradicating both cefiderocol-resistant and cefiderocol-susceptible gram-negative pathogens. Furthermore, Zid acts synergistically with cefiderocol in leading to shrinkage of the bacterial cell membrane and eliminating intracellular bacteria *in vitro*. Transcriptional analysis confirms that the combinational use of Zid and cefiderocol could cause the inhibition of key respiratory chain enzymes and efflux gene expression.

**Conclusions:** We identifed Zid restored the antibacterial effects of FDC against FDC resistant Gram-negative strains *in vitro*. Transcriptome analysis indicated that the combinational use of Zid and FDC could inhibit the expression of the vital respiratory chain enzymes, which might lead to the aberrant bacterial respiration and cell death.

## P-125

# Use of essential oils in managing ESBL *E.coli* colonization in broiler chicks: a nature-based solution to mitigate antimicrobial resistance

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**Background and objectives:** Antibiotic use and antimicrobial resistance (AMR) in poultry farming are global health issues. Essential oils from thyme and rosemary have shown antimicrobial and growth-promoting potential, making them promising alternatives to antibiotics. This study evaluated their effects on growth performance and bacterial colonization in broiler chicks infected with ESBL-producing *E. coli*.

**Methods:** One-day-old Arbor Acres chicks (n=140) were separated into four groups (CN, A, T, R) of 35 chicks depending on the treatment they will receive. Upon arrival (t1) at the livestock facility, all animals were weighted and the presence of Enterobacterlaes producing an extended-spectrum beta-lactamase (ESBL) was checked. On day 3, all chicks, except those in the negative control (CN) group, were individually infected orally with the ESBL-producing *Escherichia coli* R56. Starting on day 5, treatments were administered in drinking water as follows for five consecutive days: group A received

antibiotics (colistin + amoxicillin), group T received thyme essential oil, and group R received rosemary essential oil. Body weight gain was measured on days 17, 25, and 36. A final cloacal swab was taken individually on day 36.

**Results:** At t1, all chicks were free from ESBL-*E. coli*. Eighty-four hours after infection, the percentage of colonized chickens varied from 2.9% in the R group, to 8.6% in the A group and 17% in the T[HM1] group. At day 36, the highest percentage of colonization was observed in the A group (68%), compared with the T (5.9%) and R (6.5%) groups ($p = 10^{-3}$). Colonization was confirmed to be caused by *E. coli* R56. The average bacterial count on day 36 was significantly lower in the T group (100.00) compared with the A group (317.14), but not compared with the R group (210.00). The CN group remained free from resistant bacteria throughout the experiment. At day 36, the average body weight recorded in the R (1477.55 g) and T (1441.60 g) groups was significantly higher than in the A group (1320.97 g). The lowest average body weight was recorded in the CN group (1261 g). Mortality rates between the four groups were not statistically different.

**Conclusions:** The use of antibiotics resulted in an average body weight higher than that of standard breeding; however, the selection pressure imposed promoted the dissemination of the ESBL-producing *E. coli* R56. Conversely, treatment with thyme and rosemary essential oils provided a dual benefit, by improving the average body compared to antibiotic-treated animals, and by reducing the percentage of animals carrying ESBL-*E. coli*. These approaches are thus credible alternatives to decrease antibiotic use and antibiotic resistance in poultry production, a sector that is often regarded as an important contributor to the One Health AMR burden.

## *t06 - Novel methods and tools dedicated to antimicrobial resistance (detection, diagnostics, surveillance)*

### P-126

## Validation of the VERI-5® System for Rapid and Accurate Detection of Animal Isolates from Clinical Urine Samples Using NANOPLEX® Technology

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**Background and objectives:** Accurate and rapid detection of urinary tract infections (UTIs) is critical for effective treatment, patient care and antimicrobial stewardship. The VERI-5® system, an innovative diagnostic platform utilising NANOPLEX® technology, detects Escherichia coli (ESCCO), Klebsiella pneumoniae (KLEPN), Proteus mirabilis (PROMI), and Enterobacter cloacae (ENTCLO) from clinical urine samples. This study aimed to evaluate the system"s performance in detecting animal isolates by assessing two key parameters:

1) Specificity – the ability of VERI-5® to correctly classify negative urine samples with a target specificity of 90%.

2) Sensitivity – the ability to detect the four target species, with a target sensitivity of 90%.

**Methods:** Urine samples and clinical isolates were obtained from Axiom Veterinary Laboratories Ltd (Devon, England). Sensitivity was assessed using 82 clinical isolates (E. coli: 28, P. mirabilis: 26, K. pneumoniae: 25). E. cloacae was not tested due to low prevalence. Culture-negative urine was filter-sterilised, pooled and spiked with an individual clinical isolate, ranging from 105 – 108 CFU/mL. The

VERI-5® workflow, including filtration, buffer-exchange and direct cartridge loading, was then performed. To assess specificity, 123 culture-negative urine samples were processed directly and underwent the VERI-5® workflow. The assay employs proprietary image analysis software to correlate agglutination patterns to the presence of bacteria. Each sample was tested in triplicate, and bacterial identification and microbial load calculations were performed, with contaminated or invalid samples excluded. Data analysis was then performed to calculate the sensitivity and specificity of each species. Table 1: Sensitivity and Specificity results for each species tested on the VERI-5® system. TP = True Positives, FN = False Negatives, TN = True Negatives, FP = False Positives.

**Results:** The VERI-5 system achieved an overall sensitivity of 94%, surpassing the 90% target. At a species level, ESCCO and PROMI exceeded the 90% target, both at 96%, whilst KLEPN was slightly below target at 88%. In terms of specificity, an average of 88% was achieved, only 2% below the 90% target. 6 out of the 14 false positives were from the PROMI probe.

**Conclusions:** The VERI-5® system demonstrated excellent sensitivity, with three of the four target species exceeding the 90% target, confirming the ability of the system to accurately detect E. coli, K. pneumoniae and P. mirabilis from urine samples. Specificity fell slightly below target at 88%, mainly due to an increased number of false-positive PROMI results. This result still supports the system"s high accuracy in distinguishing positive from negative samples. These findings highlight the VERI-5® system"s potential as a rapid and reliable tool for UTI detection, leading to faster clinical decision-making and improved patient outcomes.

**Figure**

| | ESCCO | KLEPN | PROMI | ENTCLO |
|---|---|---|---|---|
| TN | 98 | | | |
| FP | 2 | 2 | 6 | 4 |
| **Specificity** | **88 %** | | | |
| TP | 27 | 25 | 25 | - |
| FN | 1 | 3 | 1 | - |
| **Sensitivity** | **96%** | **88%** | **96%** | - |

## P-127

# Evaluating the limitations of Illumina and Oxford Nanopore sequencing for dairy cattle milk resistome profiling

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**Background and objectives:** Mastitis remains one of the most important diseases in dairy cattle and a major reason for antimicrobial use. The dry period is critical for udder recovery and the selective use of antibiotics in dry-cow therapy (DCT) is common practice for mastitis control. However, growing concerns regarding antimicrobial resistance call for effective monitoring tools. The milk resistome, defined as the collection of antimicrobial genetic determinants of resistance present in milk-associated microbial communities, could be a suitable marker for assessing the impact of DCT. Illumina shotgun metagenomic sequencing has been used for resistome characterisation, but the short reads produced limit the ability to link ARGs to specific microbial hosts. To investigate the impact of antibiotic DCT on the milk resistome of dairy cattle, we compared the performance of Illumina short-read sequencing and Oxford Nanopore Technologies (ONT) long-read sequencing to evaluate whether ONT could overcome Illumina assembly and microbial host-assignment limitations.

**Methods:** Milk samples from 31 clinically healthy Holstein cows (18 treated with antimicrobial DCT (T) and 13 untreated (NT)) were subjected to Illumina shotgun metagenomic sequencing (2 × 150 bp). Ten of these samples (5 T, 5 NT) were sequenced on the ONT MinION Mk1C platform. Illumina and ONT sequences were analysed with abricate, both queried against the MEGARes database. Illumina contigs and ONT reads carrying ARGs were further analysed by BLAST for taxonomic assignment.

**Results:** Illumina generated an average of 3.9 Gb/sample ($N50_{assembly}$ = 850 bp; average Q = 38.1). Twenty-five ARGs corresponding to eight antimicrobial classes were detected but no reliable taxonomic assignment could be established of ARG-harbouring contigs. ARGs were identified in 50.0% of T and 46.2% NT, with T animals exhibiting greater resistome diversity (21 ARGs, 8 classes) compared to NT (13 ARGs, 3 classes). ONT sequencing yielded an average of 1.5 Gb/sample ($N50_{reads}$ = 6,500 bp; average Q = 30.6). ARGs were only detected in four T cows, accounting for a total of 11 ARGs (4 classes). Confident host assignment was achieved for three ONT reads. Illumina analysis of the same ten samples identified 6 ARGs, three of them also identified by ONT. In both sequencing approaches ARGs associated with macrolide-lincosamide-streptogramin and aminoglycoside resistance predominated, while genes conferring resistance to β-lactams and sulphonamides were also detected but less frequently. In both cases, only 10% of reads were of microbial origin after host DNA removal.

**Conclusions:** Resistome analysis of low-biomass, host-DNA rich matrices such as bovine milk by either Illumina or ONT sequencing remains a challenge. Although reads produced by ONT were longer than Illumina contigs, it did not significantly enhance ARG detection or reliable taxonomic linkage of ARGs. Prevalence and diversity of ARGs was consistently higher in T than NT animals regardless the sequencing method used, suggesting that antibiotic use in DCT alters the resistome of bovine milk. The low ARG abundance detected is reassuring but suggests that resistome characterisation in milk may require enrichment-based strategies such as ARG-targeted capture sequencing or ONT adaptative sampling.

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## P-128

# Towards Better Control of Antibiotic Resistance in Human and Animal Health in Togo

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**Background and objectives:** This study, carried out in Togo, addresses the growing problem of bacterial resistance to antibiotics. Without precise data on the region's multi-resistant bacteria (MRB),

our study aims to characterize these strains and identify transmission routes to better understand their spread. The aims of our study were i) to estimate the prevalence of digestive carriage of e*xtended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E*) and carbapenemase-producing Enterobacteriaceae (CPE) among livestock farmers and their animals living in the same locality as patients carrying MRB; ii) identify the risk factors that have contributed to the emergence of *ESBL-E* and CPE.

**Methods:** Farmers in maritime areas with a high risk of antimicrobial resistance (AMR) emergence were targeted. Sampling was based on the assumption that at-risk areas had a high probability of identifying MRB in humans. Thus, our survey began with the identification of MRB-carrying patients diagnosed and managed in the orthopedic, intensive care, pediatric, and gynecological departments of Lomé's two main university hospitals: Sylvanus Olympio and Campus de Lomé. Two to three farms located within 2 km of the patients' homes were then selected for the study. Samples were taken from these farmers and their animals for in-depth analysis of resistant bacterial strains.

**Results:** Prevalence analyses, although the evaluation of occurrence factors is still in progress, revealed a high rate of ESBL-E and CPE carriage in the different animal productions. Poultry production showed a carriage rate of 93% for ESBL-E and 32% for CPE. Pig production showed 100% carriage for EBLS-E and 11% for CPE. In small ruminants, carriage rates were 100% for ESBL-E and 57% for CPE, while in cattle they were 100% for ESBL-E and 14% for CPE. ESBL-E strains isolated from livestock showed high rates of antibiotic resistance, including 88% to Cefotaxime, 88% to Cefepime, 71% to Ceftazidime, 69% to Amoxiclav, 45% to Ertapenem, 21% to Temocillin and 2% to Imipenem. The majority of these strains belonged to *Escherichia col*i (82%), followed by *Klebsiella spp*. (6%). The ESBL-E carriage rate among farmers, was 92%, while the CPE carriage rate was 23%. The ESBL-E strains found in the latter were 87% resistant to Cefotaxime, 85% to Cefepime, 79% to Ceftazidime, 60% to Amoxiclav, 45% to Temocillin and 12% to Ertapenem. *Escherichia coli* was the predominant strain (53%), followed by *Serratia spp*. (13%).

**Conclusions:** Although prevalence data for ESBL-E carriage on farms is alarming, it is remarkably rare to detect CPE in animals, given that carbapenems are considered antibiotics of last resort, reserved primarily for treatment in hospitals, particularly intensive care units. An increase in the presence of CPE in livestock, and even in breeders, could lead to higher mortality and longer hospital stays. It is therefore imperative to take measures to limit the spread of these bacteria on farms. Restricting the use of antibiotics is essential to reduce the pressure on the selection of resistant bacterial strains.

## P-129

# Emerging Threat of Antimicrobial Resistance in Poultry from the Pakistan-Afghanistan Border: Insights into ESBL and Carbapenem-Resistant Enterobacteriaceae

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**Background and objectives: Background:** Surveillance of antimicrobial resistance (AMR) in the animal health sector is critical for assessing the spread of resistant pathogens. This study employed active surveillance to monitor Extended-Spectrum Beta-Lactamase (ESBL) and Carbapenem-Resistant Enterobacteriaceae (CRE) Escherichia coli in healthy poultry and passive surveillance to detect Escherichia coli, Salmonella spp., and Staphylococcus aureus in sick poultry.

**Methods: Methods:** A total of 500 samples (350 cloacal swabs from healthy birds for active surveillance, 150 samples from sick birds for passive surveillance) were collected from 50 poultry farms across five districts of Pakistan-Afghanistan Border. Bacterial isolation and antimicrobial susceptibility testing were performed using standard disk diffusion methods. The presence of ESBL and CRE was confirmed phenotypically. Risk factors were assessed through structured farm surveys.

**Results: Results:** ESBL-producing E. coli was detected in 38% (133/350) of healthy poultry samples, while CRE isolates were found in 12% (42/350). Among sick birds, E. coli (60%), Salmonella spp. (25%), and Staphylococcus aureus (15%) were isolated, with 75% of E. coli isolates showing multidrug resistance (MDR).Antibiotic resistance patterns revealed high resistance to cephalosporins (85%), fluoroquinolones (72%), and aminoglycosides (50%), while colistin resistance was observed in 5% of isolates. risk factors associated with AMR included frequent antibiotic use (OR: 4.2, $p < 0.001$), poor farm hygiene (OR: 3.5, $p = 0.002$), and lack of biosecurity measures (OR: 2.8, $p = 0.005$).

**Conclusions: Conclusion:** This study underscores the widespread presence of antimicrobial-resistant bacteria in poultry farms of Pakistan-Afghanistan Border. The findings highlight the urgent need for strengthening AMR surveillance, enforcing judicious antibiotic use, and implementing biosecurity measures to mitigate the spread of resistance in both healthy and sick poultry populations.

## P-130

# Evaluation of CLSI- and EUCAST-Approved Quality Control Strains for Antimicrobial Susceptibility Testing of *Mycoplasma hyorhinis*

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**Background and objectives:** Antimicrobial susceptibility testing (AST) should be conducted in a standardized manner prior to the start of an antimicrobial treatment. For fastidious bacteria, such as porcine *Mycoplasma* (*M.*) spp., specifically *M. hyorhinis*, neither guidelines or standards for the performance of AST, nor quality control (QC) strains for the validation of AST results are approved by organizations like the Clinical and Laboratory Standards Institute (CLSI) or the European Committee of Antimicrobial Susceptibility Testing (EUCAST).

**Methods:** The CLSI- and EUCAST-approved quality control strains *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 were chosen to validate AST by broth microdilution using modified Friis broth, developed as growth medium for porcine *Mycoplasma* spp. like *M. hyorhinis*. The antimicrobial agents doxycycline, enrofloxacin, erythromycin, florfenicol, gentamicin, marbofloxacin, tetracycline, tiamulin, tilmicosin, tulathromycin, and tylosin were examined using customized SensititreTM microtiter plates.

**Results:** *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 were tested in modified Friis broth for 24, 48, and 72 h at 14 independent occasions. Despite the fact, that a different test medium and prolonged incubation times were used, the minimal inhibitory concentrations (MICs) obtained after these incubation times for *E. faecalis* ATCC 29212 were mostly within the CLSI-approved quality control ranges for the following antimicrobial agents: doxycycline, enrofloxacin, erythromycin, florfenicol, gentamicin, tilmicosin, tulathromycin, and tylosin. For *S. aureus* ATCC 29213, the MICs obtained under the same conditions were mostly within the CLSI-approved QC ranges for clindamycin, marbofloxacin, tetracycline and tiamulin [1]. Solely 7/168 (4.2%) of the MIC values were one concentration step above the CLSI-approved QC ranges, with 6/7 MIC values noted after 72 h of incubation for clindamycin, enrofloxacin, marbofloxacin, and tetracycline [1].

**Conclusions:** These results showed that the two widely used QC strains *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 produced MIC values in modified Friis broth after prolonged incubation times that were largely within the CLSI-approved QC ranges for MIC values in cation-adjusted Mueller

Hinton broth and shorter incubation times. Hence, they might be used for QC in AST of *M. hyorhinis* isolates until more suitable species-specific QC strains are identified and approved.

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## P-131

# International Efforts to Support Local Actions on Antimicrobial Resistance

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**Background and objectives:** Antimicrobial Resistance (AMR) represents a significant One Health issue, posing a substantial threat to public and animal health, the environment, and the global economy. Effective mitigation of this threat necessitates a comprehensive approach encompassing human health, animal health, agriculture, and environmental sectors. The adverse impacts of AMR are anticipated to be most severe in Low- and Middle-Income Countries (LMICs), where national data on AMR in non-human sectors are often limited, surveillance capacity is low, and regulatory policies for medicines are underdeveloped. Since its designation in April 2019, the UK"s FAO Reference Centre for AMR has been instrumental in supporting AMR action through expert consultancy, capacity development, and the facilitation of international engagement and cooperation.

**Methods:** To achieve our objectives, we align our efforts with FAO"s programme by assisting member countries in implementing their National Action Plans on AMR. Our approach is rooted in partnership, collaborating directly with laboratory, policy, and regulatory counterparts to understand their unique challenges and tailor our support accordingly. We engage with a diverse range of partners to deliver activities across four broad categories: international engagement, capacity building, surveillance and research, and the provision of guidance and standards. Our primary focus remains on building capacity for long-term sustainability and resilience.

**Results:** We have facilitated international engagement through meetings and workshops, including hosting the inaugural Congress of FAO Reference Centres for AMR, attended by FAO colleagues and Reference Centre heads from eight countries. Our AMR Community of Practice, comprising over 90 experts from 25 countries, promotes good laboratory practices and professional networking. Since our designation, we have supported capacity development through training at our UK facilities (29 visitors from 9 countries) and at our partners" facilities (>350 scientists trained in 9 countries). We provide mentorship for the Fleming Fund Fellowship programme and support AMR-related postgraduate programmes.

**Conclusions:** Collaborative projects with partners in various countries have enhanced research capacity and generated quality data addressing AMR-related evidence gaps. We support good laboratory practices and quality data generation through AMR Proficiency Testing schemes. Our experts have contributed to key guidance and standards, such as susceptibility testing protocols and FAO Regional AMR Guidelines. These activities have supported the implementation of AMR Action Plans at both global and country levels, enabling a holistic One Health approach that complements and integrates into existing action plans. Working internationally to support local action on antimicrobial resistance.

**P-132**

# *Vibrio* wound infections: Antibiotic Therapies and Resistance in a Changing Environment

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**Background and objectives:** *Vibrio* infections are generally associated with the ingestion of contaminated water and seafood. *Vibrio* species also cause wound infections, which can be difficult to diagnose, progress rapidly, and prove fatal. The incidence of human infections is increasing, due to higher sea surface temperatures, decreased salinity and increased storm events, with a significant increase in infections reported in Europe [1] and the USA [2]. There is clearly a need for a more coordinated approach to establishing which treatments are appropriate for *Vibrio* wound infections, particularly given the predicted increase in antimicrobial resistance.

**Methods:** We analysed over 30 years of data from the USA"s Cholera and Other *Vibrio* Illness Surveillance (COVIS) system focussing on wound infections, *Vibrio* species, antibiotic treatment, antibiotic class and clinical outcome. We also collated recent epidemiological data from across Europe from peer-reviewed literature, grey literature etc, to assess the prevalence of infections in Europe. We reviewed data from peer-review studies across Europe, to assess to prevalence of antimicrobial resistance (AMR) in various *Vibrio* species of clinical concern.

**Results:** Of 28,500 COVIS entries, 5,000 were wound infections, *V. alginolyticus* caused the most cases (1,564), but *V. vulnificus* accounted for more fatalities (166 of 1009 cases (16.5%)). Doxycycline (tetracycline) was prescribed in 1,260 cases, followed by ciprofloxacin (quinolone) and sulfamethoxazole-trimethoprim (sulfonamide & dihydrofolate reductase inhibitor), 860 and 637 cases respectively. Fatality rates in *V. vulnificus* infections for doxycycline treatment 8.4% (n=95), ciprofloxacin 4.9% (n=61) and sulfamethoxazole-trimethoprim 0% (n=21). There was a significant decline in overall fatality rates in all *Vibrio* wound infections from 2005 onwards.

**Conclusions:** Several studies on *Vibrio* isolates from seafood and food-borne infections in Europe have revealed a broad range of AMRs, some of which are intrinsic. The most frequently reported AMRs for *V. parahaemolyticus* include ampicillin (70%–100% in seven studies) and streptomycin (30%–70% in six studies). For non-O1/non-O139 *V. cholerae*, resistances to colistin (87%–100% in four studies), ampicillin (4%–75% in five studies), and streptomycin (11%–68% in four studies) have been observed. Additionally, antimicrobial resistance genes (ARGs) linked to mobile genetic elements, which confer resistance to various β-lactams, quinolones, sulfonamides, aminoglycosides, tetracyclines, folate pathway inhibitors, and phenicols, have been identified in a good spread of *Vibrio* species.

There is a significant increase in human clinical infections associated with *Vibrio* species with data from both the USA and Europe augmenting this finding. Increased awareness of these infections is required, as well as better outreach and education programmes. The lack of a legal framework for reporting and notification of vibriosis in Europe remains an ongoing issue, and so too the lack of recommended antimicrobial treatment options.

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**P-133**

# Genome-based prediction of minimum inhibitory concentration for doxycycline in *Campylobacter jejuni*

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**Background and objectives:** Antimicrobial resistance (AMR) in foodborne pathogens like *C. jejuni*, known for its high genetic adaptability, poses a significant global health threat and the search for alternative strategies is encouraged [1]. Public sharing of whole-genome sequencing data alongside AMR metadata enables the application of machine learning to predict AMR phenotypes without relying on pre-existing databases of known AMR genes or mutations. In this work, we present a machine-learning approach to predict the minimum inhibitory concentration (MIC) of *C. jejuni* to doxycycline using whole-genome sequencing data.

**Methods:** *C. jejuni* genome sequences and corresponding AMR data were obtained from the National Antimicrobial Resistance Monitoring System [2]. Sequencing data were downloaded and processed using Slovenian National Supercomputing Network to create a pangenome and generate a binary presence-absence matrix. The Orange data mining tool [3] was used to evaluate machine learning models against the training data with 5-fold cross-validation. Lasso regularisation was applied to reduce the model complexity before testing. The most predictive features and surrounding genes were screened against CARD database [4].

**Results:** Among the tested models, linear regression performed best in predicting the MIC of *C. jejuni* for doxycycline. Cross-validation confirmed that the model was robust across different data subsets. We achieved a root mean square error within ±1 two-fold dilution factor and an R2 score of 0.946 in unseen test data. We identified key predictors, including known AMR-associated genes *tet(O)*, *tet(M)-like*, multidrug efflux transporters (*mdfA-like*), and novel candidate genomic regions.

**Conclusions:** Machine learning can predict *C. jejuni* MIC for doxycycline, identifying both known AMR determinants and novel genomic regions associated with resistance. These findings improve AMR surveillance and deepen our understanding of doxycycline resistance in *C. jejuni*, a major foodborne pathogen.

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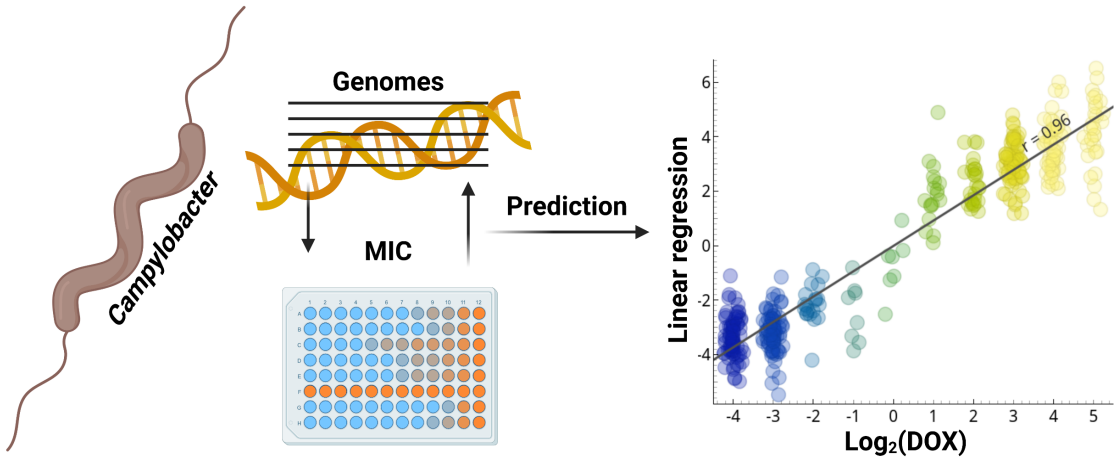
**Figure**




## P-134

# Surveillance of ESBL and CRE in the Animal Health Sector: Antimicrobial Resistance in Khyber Pakhtunkhwa

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**Background and objectives:** AMR in food animals threatens public health by spreading resistant bacteria. There has been minimal research on AMR, and only limited data is available in the animal health sector of Khyber Pakhtunkhwa. This surveillance study aimed to study ESBL and CRE producing *E. coli* in healthy birds through active surveillance and to detect resistance pattern in *E. coli and Salmonella* in sick poultry via Passive surveillance in Khyber Pakhtunkhwa.

**Methods:** A total of 609 samples (408 ceacal swabs from healthy broilers on 39 farms and 201 clinical samples of sick poultry) were analyzed. Bacterial isolation and antimicrobial susceptibility testing followed disc diffusion methods, with CRE and ESBL confirmed phenotypically. A structured questionnaire was used to collect all relevant information for assessing risk factors.

**Results:** Among healthy samples, (8.3%) were CRE positive and 34.6% were ESBL-producing bacteria. CRE isolates exhibited high resistance to cefotaxime (88.25%), ceftazidime (61.8%), ciprofloxacin (73.5%), tetracycline (88.2%), and ampicillin (94.1%). ESBL isolates were resistant to cefotaxime (80.1%), ceftazidime (77.3%) and amoxicillin-clavulanic acid (80%). Meropenem (70.2%) and impenem (83%) were the most effective. Retrospective data of sick samples had similar resistance patterns in E. coli (n=126), Salmonella (n=75) with insignificant carbapenems resistance.Risk factor analysis associated ESBL occurrence with preventive antibiotic use (OR=2.15, p=0.07) and inadequate biosecurity (OR=2.89, p=0.04). The risk of AMR was elevated by unregulated antibiotic use (OR=3.12, p=0.02), untreated water (OR=2.47, p=0.05), and poor litter management (OR=2.75, p=0.03), whereas antimicrobial stewardship training significantly reduced the risk (OR=0.58, p=0.03).

**Conclusions:** High resistance to cephalosporins and beta lactams along with ESBL and CRE presence highlights a growing AMR threat.This alarming trend emphasizes the urgent need for enhanced surveillance systems to monitor resistance patterns, promote responsible and judicious antibiotic use in veterinary settings, and implement stringent biosecurity measures at farms.

## P-135

# Validation of two rapid pen-side diagnostic tools to detect *erm*(42) and *msr*(E) in beef cattle diagnosed with bovine respiratory disease

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**Background and objectives:** Macrolide resistance among bovine respiratory disease (BRD) pathogens is most often attributed to one or more of three resistance genes: *erm*(42), *msr*(E), and *mph*(E). Detecting proteins encoded by these genes via a chute-side lateral-flow immunoassay (LFIA) can accelerate the detection of macrolide-resistant BRD pathogens, such as *Mannheimia haemolytica* and *Pasteurella multocida*, while improving antimicrobial stewardship and managing bovine respiratory disease. The primary aim of this study was to evaluate the sensitivity (Se) and specificity (Sp) of two novel LFIAs targeting erythromycin resistance methyltransferase (*erm*(42)) and macrolide and streptogramin B resistance (*msr*(E)) in nasal and nasopharyngeal swabs collected from feeder cattle diagnosed with BRD.

**Methods:** A total of 400 beef cattle were included in a case-cohort study conducted at two large U.S. beef cattle feed yards. A shallow nasal swab and a deep nasopharyngeal swab were collected from each of 200 control (pulled and/or treated for the first time) and 200 cases (pulled and/or treated two or more times) cattle, with pairs matched by pen ID. Historical data on metaphylaxis and antibiotic treatment were compiled from feed yard records. Nasal and nasopharyngeal swabs were plated on blood agar with azithromycin and erythromycin susceptibility disks, and BRD pathogens were isolated and identified. Additionally, 0.5 mL of Amies transport medium from each swab was enriched in 4.5 mL of brain-heart infusion broth (BHI+AZI) containing an azithromycin disk (15µg/mL) and spiral plated to quantify macrolide-resistant *M. haemolytica* and *P. multocida* populations. A subset of isolates (18 *M. haemolytica* and 12 *P. multocida*) from swab samples with the highest bacterial counts were selected to perform PCR for the *erm*(42) and *msr*(E) genes and compared to results from the *erm*(42) and *msr*(E) LFIAs performed on both the isolates and corresponding BHI+AZI enrichment cultures.

**Results:** Among the 400 shallow nasal and 400 deep nasopharyngeal swabs, we identified 143 and 160 *M. haemolytica* and 113 and 131 *P. multocida*, respectively. Interim results with the LFIAs showed 88% Se for *erm*(42) and 100% Se for *msr*(E) for isolates. LFIAs used with BHI+AZI enriched nasal samples showed 35% Se for *erm*(42) and 57% Se for *msr*(E) compared to PCR results. Investigations to evaluate *erm*(42) and *msr*(E) Sp are ongoing. Further phenotypic and genotypic analyses of isolates derived from target-positive and negative samples, including MIC determination and whole genome sequencing (WGS), are underway to explore macrolide resistance profiles in cultured BRD pathogens.

**Conclusions:** These findings suggest that *msr*(E) is highly prevalent in both species, whereas *erm*(42) is more prevalent in *P. multocida*. Additionally, the *erm*(42) and *msr*(E) LFIAs can detect macrolide resistance in *M. haemolytica* and *P. multocida*; however, further optimization is needed to evaluate the specificity of these tools and to detect these targets from enriched cultures versus directly from cattle nasal samples.

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# Investigating the Role of the DUF445-Containing Putative Membrane Protein in Albicidin Resistance in *Acinetobacter baumannii* IMT51508

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**Background and objectives:** Antimicrobial resistance (AMR) has emerged as a critical global health challenge impacting all living beings. According to a recent report, AMR was the major cause of causalities approximately 5 million deaths worldwide in 2019. However, projections suggesting up to 10 million deaths annually by 2050. [1]. Albicidin, a promising antibacterial peptide, functions by inhibiting the activity of bacterial DNA gyrase [2]. While extensively studied in *Salmonella Typhimurium* and *Escherichia coli*, the known resistance mechanisms to albicidin include its degradation by the endopeptidase AlbD and binding by the MerR-like transcriptional regulator AlbA [3]. This study aims to elucidate the mechanisms underlying albicidin resistance in ESKAPE pathogens, which are leading contributors to nosocomial infections. Specifically, we focus on investigating the resistance mechanisms in *Acinetobacter baumannii* (IMT51508), a clinically significant multidrug-resistant pathogen.

**Methods:** The Minimum Inhibitory Concentration (MIC) of albicidin was determined using broth microdilution method, followed by laboratory evolution, where the albicidin concentration was incrementally increased by two-fold. The successful evolution of bacterial strains was confirmed by MIC assays of all independently evolved replicates. Subsequently, genomic DNA was extracted from the wild-type strain and eight evolved strains, and whole genome sequencing (WGS) was performed. Bioinformatics analysis was employed to analyze YjiN protein, suggesting its role as a 2-3 transmembrane domain containing protein. To further elucidate the operon structure of gene cluster, qRT-PCR was conducted by designing the interagenic and intergenic primers. In addition, mutations in YjiN and MATE efflux transporter in *Acinetobacter baumannii* AYE-T were performed, and their effects on albicidin sensitivity were measured through MIC assays. The mutation of the YjiN protein in IMT51508 is awaiting completion.

**Results:** Genome analysis revealed a consistent mutation in an uncharacterized protein, containing a DUF445 domain, YjiN, in 7 out of the 8 evolved strains. Bioinformatics analysis was employed to analyze YjiN protein, suggesting its role as a 2-3 transmembrane domain containing protein. qRT-PCR results analysis suggested that hypothetical protein is part of an operon containing DUF924 domain containing protein, Lipoprotein, and aspB, while MATE is not part of the operon. The MIC of AYE-T-ΔYjiN and AYE-T-ΔMATE is increased by two-fold.

**Conclusions:** The involvement of the YjiN protein in *Acinetobacter baumannii* IMT51508 will provide valuable insights into the role of this putative membrane protein in the bacterial resilience to albicidin. Understanding its involvement will enhance our comprehension of the mechanisms contributing to albicidin resistance in *Acinetobacter buamannii*.

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# Development of a Multiplex ddPCR Technique for detection of *bla*$_{OXA-48}$-positive *K. pneumoniae* in wastewater samples

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**Background and objectives:** Infectious diseases and the associated antimicrobial resistance (AMR) represent a global threat to human health. Carbapenemase-producing Enterobacteriaceae are listed as critically important pathogenic bacteria by the World Health Organization (WHO). One representative is Klebsiella pneumoniae, a species that is known for its increasing resistance to different antimicrobial agents. In industrialized countries, systematic disease surveillance systems are in place to detect the occurrence and spread of critical infectious diseases. To complement the classic clinical and syndromic surveillance systems, wastewater-based epidemiology (WBE) detects targets of interest in raw influent wastewater. Thus, WBE offers an opportunity to include target information representing all people of a specific population connected to a wastewater treatment plant (WWTP). Since culture-based approaches are time-consuming and costly, a molecular approach for detecting and quantifying AMR in wastewater would be desirable. To reach this goal, the challenges associated with analysis of pathogen-resistance combinations need to be overcome.

**Methods:** In this study, a multiplex droplet digital PCR (ddPCR) was established to detect carbapenemase-producing *K. pneumoniae*. For this purpose, an assay with two primer-probe systems targeting species-specific genes and a third primer-probe system for detecting the carbapenemase gene *bla*$_{OXA-48}$ was used. Wastewater samples were used as templates without prior processing.

**Results:** It was shown that the detection of *K. pneumoniae* in a pure culture and in wastewater was feasible. However, the number of positive droplets was overall close to the detection limit. Besides, some reactions failed to generate enough droplets for analysis.

**Conclusions:** Further optimization of the approach is needed, e.g., by filtering the wastewater to remove larger particles in putative PCR-inhibiting substances. With further development, a multiplex ddPCR might offer a possibility to provide fast quantification of pathogen-resistance combinations of interest in wastewater samples.