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Abstract Book

O01
Identification of non-essential genes required for intrinsic resistance to macrolides in *Escherichia coli* by Transposon Directed Insertion-site Sequencing (TraDIS)

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Introduction:

Enterotoxigenic *Escherichia coli* (ETEC) is the main cause of post-weaning diarrhoea (PWD) in pigs. Macrolides are widely used in pig farming but not for treatment of ETEC PWD due to intrinsic resistance of *E. coli* to this antimicrobial class. In this study, we investigated chromosomal non-essential genes involved in intrinsic resistance of *E. coli* to tilmicosin, a macrolide licensed for the treatment of pig enteritis.

Methods:

We constructed two high-density transposon mutant libraries of >290,000 and >390,000 unique Tn5 insertions in a clinical (ETEC5621) and a laboratory (K-12 MG1655) *E. coli* strain, respectively. We measured mutant depletion upon exposure to two sub-minimal inhibitory concentrations (sub-MIC) of tilmicosin by Transposon Directed Insertion-site Sequencing (TraDIS).

Results:

TraDIS analysis identified genes required for growth of ETEC5621 and MG1655 during exposure to 1/8 MIC (n=15 and 16, respectively) and 1/4 MIC (n=38 and 32, respectively) of tilmicosin. In both strains, all genes identified after exposure to 1/8 MIC were identified following exposure to 1/4 MIC. Ten genes were found essential for growth under 1/8 MIC of tilmicosin in both ETEC5621 and MG1655, including genes involved in outer membrane integrity (n=6), multidrug efflux pump (n=3) and glucose metabolism (n=1). Thirteen additional genes were required for growth under 1/4 MIC condition in both strains, including genes related to cell membrane (n=11), gene encoding ribosomal protein (n=1) and gene encoding RNA helicase (n=1).

Conclusions:

Upon further validation, the genes identified in our study can be exploited as drug targets to re-sensitize ETEC and other pathogenic *E. coli* to macrolides.

O02

Potential for zoonotic spread of multi-resistant *Clostridioidies difficile*

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Background: *Clostridioides difficile* is a spore-forming pathogen causing severe enteric infections. Sequence type 11 (ST11), with its main PCR ribotype 078 (RT078) is the second most common clinical type in Denmark associated with a rising number of infections in the community. Farm animals have been identified as RT078 reservoirs. By whole genome sequencing (WGS) of veterinary and human bacterial isolates, we investigated the potential for zoonotic spread and associated antibiotic resistance genes (ARG).

Methods: A collection of samples was established during 2020-22: pig farms (A, n=330), slaughterhouses (B, n=184), chicken farms (C, n=171). After screening, positive *C. difficile* isolates were subjected to WGS (Illumina). Additionally, thirty-one isolates collected during 2010-12 from meat (n=31) were included. Whole genome MLST (wgMLST, 8600 loci) and AMRfinder were used to compare the veterinary isolates to Danish clinical isolates from the last four years.

Results: *C. difficile* was found in 44 samples (A= 29, B= 7, C=4); adding the meat samples, the collection consisted of 75 isolates. Twelve sequence types (ST) were shared between animals and humans (n= 1894). Five STs included isolates within possible transmission range (<3 wgMLST alleles, no. in brackets): ST3(3), ST6(1), ST11(22), ST16(1), ST14(1). The veterinary ST11 clustered with several different human subgroups and shared identical ARGs including: β-lactams, aminoglycosides, fluoroquinolones and tetracycline (Figure 1).

Conclusions:
ST11 is among one the most resistant *C. difficile* in the clinical and veterinary environments. The close similarly of veterinary and human isolates indicates possible transmission and exchange of ARGs between the two environments.

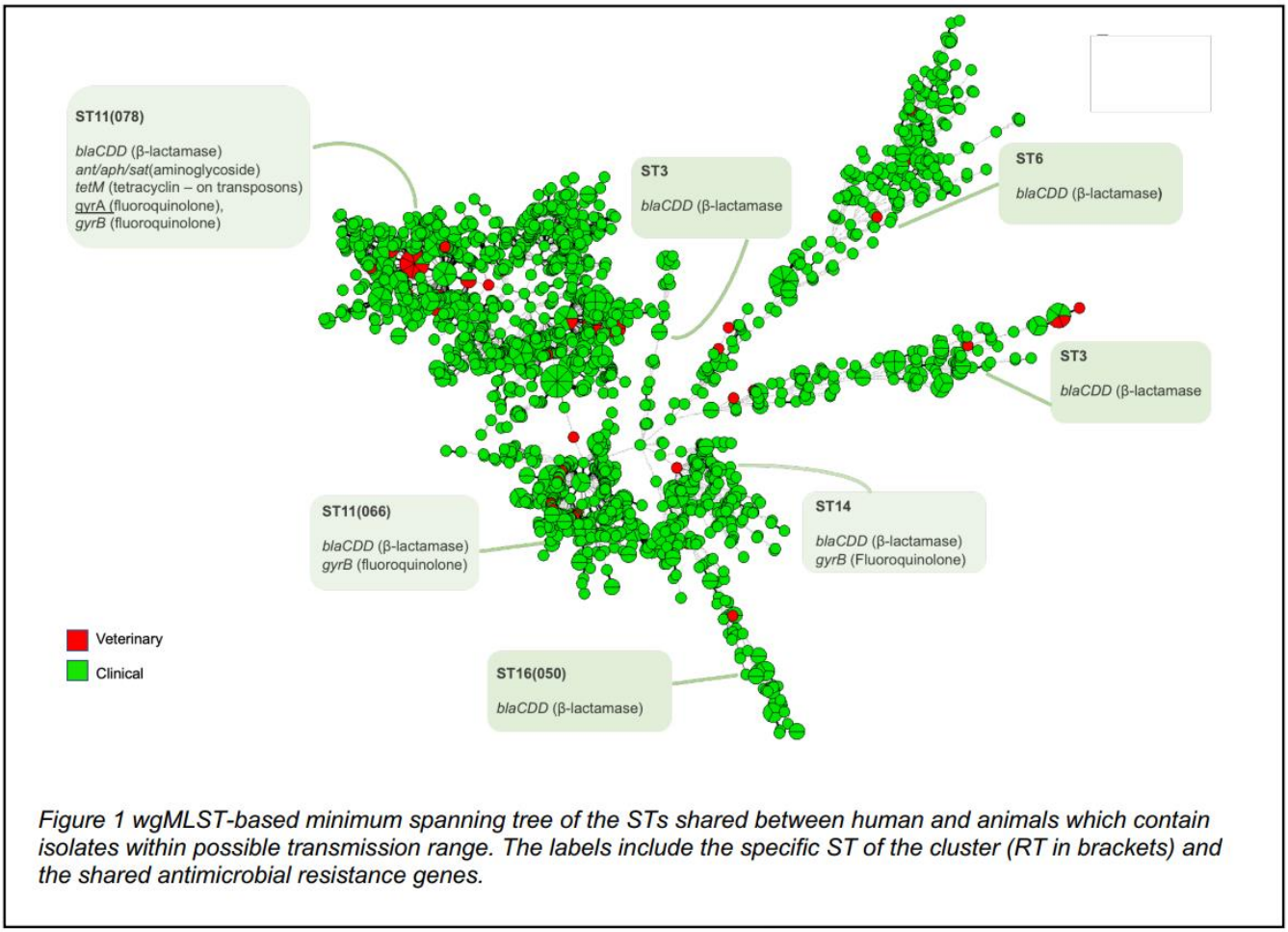

Figure 1 wgMLST-based minimum spanning tree of the STs shared between human and animals which contain isolates within possible transmission range. The labels include the specific ST of the cluster (RT in brackets) and the shared antimicrobial resistance genes.

O03
Evaluation of β-lactamase enzyme activity in Outer Membrane Vesicles (OMVs) isolated from Extended Spectrum β-lactamase (ESBL) *Salmonella* Infantis strains

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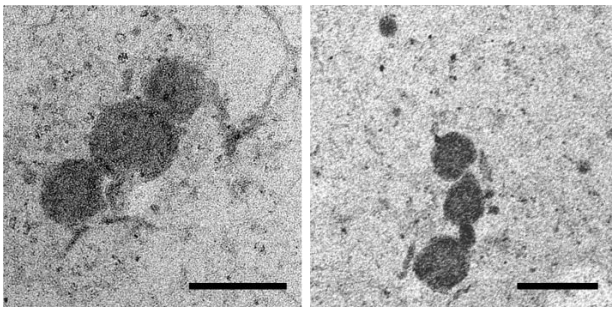
Introduction:
Recent studies have shown that Outer Membrane Vesicles (OMVs), released by Gram-negative bacteria are involved in antibiotic-resistance (AR) mechanisms by including β-lactamase enzymes. Since no studies have been conducted on *S*. Infantis' OMVs yet and, in the last years, multidrug resistant and extended-spectrum beta-lactamases (ESBLs) *Salmonella* Infantis strains have spread widely in Europe, the aim of the work was to collect OMVs from five *S*. Infantis β-lactam resistant strains isolated from a broiler meat production chain and to investigate whether β-lactamase enzymes are included in OMVs during their biogenesis.

Methods:
OMVs were isolated by ultrafiltration. The β-lactamase activity was quantified by Nitrocefin assay in three different samples: the filtered supernatants, the eluted samples from ultrafiltration, and then OMVs concentrates. Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) were used to identify the OMVs.

Results:
The results showed that all strains release spherical OMVs, ranging from 60 to 230 nm (Fig.1)



The Nitrocefin assay highlighted the presence of β-lactamase enzymes within the OMVs. The β-lactamase enzymatic activity in OMV concentrates showed significantly higher values when compared with 0.45 filtrate and eluted samples, respectively. No statistically significant differences were observed between the 0.45 filtrate and eluted samples.

Conclusions:
Our results suggest that β-lactamase enzymes also get packaged into OMVs from bacterial periplasm during OMV biogenesis. A possible explanation could be that enzymes are loaded into OMVs to be protect from proteases degradative action. An investigation into the possible role played by OMVs in AR mechanisms would open the door for an opportunity to develop new, therapeutic strategies.

O04
How to fight antimicrobial resistance (AMR) through the development of autogenous veterinary vaccines

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Introduction:

The use of antibiotics in animal production, terrestrial and aquatic fields, represents one of the most critical aspects of modern farms. It is undeniable that international guidelines strongly recommend a significant reduction in the use of antibiotics in all livestock production chains. At the same time it is equally clear and true that human health, animal health and ecosystem health are linked as proposed by the one health approach.

Methods:

Several new mono or polyvalent autogenous veterinary vaccines based on different antigens (bacteria, viruses, fungi, etc.) have been developed and administered using new production technologies, new adjuvants and new immunostimulant and immunomodulant molecules. Development and validation of new vaccines is essential to contrast animal disease and is essential to reduce the use of antibiotics and successfully counteract antimicrobial resistance (AMR).

Results:

Several new autogenous vaccines have been developed and administered in various animal species with excellent results in preventing (prophylaxis) and sometimes in treating (metaphylaxis) emerging or re-emerging diseases and the new pharmaceutical technologies have been able to guarantee both small and large productions of vaccines also in the veterinary field for both terrestrial and aquatic animals.

Conclusions:

the "one health" approach provides a health model based on the integration of different disciplines and skills as an essential resource for achieving global health; veterinary vaccines certainly represent a possible way forward to effectively combat the phenomenon of antimicrobial resistance and ensure public health. This Research is carried out with the funding of the Italian Ministry of Health - IZSUM112021RC.

O05
Applying Implementation Mapping: developing the intervention program for the *Streptococcus suis* clinical practice guideline in weaned pigs

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Introduction:

Streptococcus suis (S. suis) infections are the cause of substantial consumption of antimicrobials in pigs. In the Netherlands, the veterinary clinical practice "*S. suis* in weaned pigs guideline" was published online in 2014 but no additional activities were undertaken to support the uptake and implementation. Adherence of veterinarians to the *S. suis* guideline can improve antimicrobial stewardship as it includes recommendations about prevention of *S. suis* and on antimicrobial therapy. However, a survey showed that the *S. suis* guideline was not or only partly used. We present the development of an evidence-based intervention program to implement the *S. Suis* guideline.

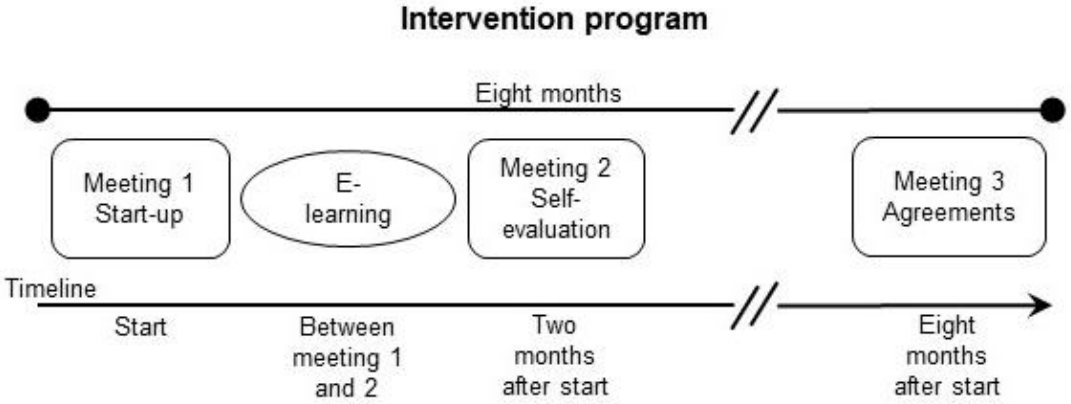
Methods:

We used Implementation Mapping, to systematically develop an intervention program following five tasks. This tasks included qualitative research to analyze the implementation gap which we used to analyze objectives and design the intervention program.

Results:

We developed an intervention program for practicing swine veterinarians which consists of three peer group meetings and an e-learning module, see Figure 1. In the intervention we used multiple behavior change techniques such as reflecting on their own prescription behavior and providing evidence based literature in a convenient way. During the intervention, outcome and process indicators were measured such as the antimicrobial use at farms and the attitude of participants towards the *S. suis* guideline.

Figure 1 Intervention Program Abstract ICOHAR 2023



Conclusions:

The Implementation Mapping approach was helpful to ensure a complete analysis was done and an argumentation is present for every decision regarding the development of the intervention.

P01
High-level and within-host diversity of resistomes in coagulase-negative staphylococci: First detection of optrA- and cfr-carrying strains in healthy pigs and pig-farmers in Spain

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Introduction:

This study characterized the mechanisms of antimicrobial resistance (AMR), within-host and intra-species resistomes diversity in a collection of coagulase-negative staphylococci (CoNS) recovered from nasal cavities of healthy pigs and pig-farmers.

Methods:

One-hundred-and-one CoNS isolates (9 species) recovered from 40 pigs and 10 pig-farmers of four Spanish pig-farms and identified by MALDI-TOF-MS were tested for their AMR phenotype. Non-duplicate isolates (from different individuals, species, or AMR phenotypes) were selected (n=85) and their AMR genotypes were characterized by PCR/sequencing.

Results:

A total of 101 CoNS (9 species) were identified from 72.5% and 60% of pigs and pig-farmers, respectively. Of the 85 non-duplicated isolates, only 1 (1.2%) S. chromogenes was susceptible to all antibiotics tested, 90.5% showed a multidrug resistance (MDR) phenotype, and 44.7% were mecA-positive. About 40.7% of the pigs and pig-farmers had within-host (> 1 CoNS species in a host) while 55.6% had intra-species AMR diversity (same species with 2-5 profiles). The following AMR were detected among the non-duplicate CoNS (percentage of isolates/ genes detected): tetracycline (94.1/tetK, tetL, tetM, tetO), penicillin (72.8/blaZ), erythromycin-clindamycin-constitutive (77.6/ermA, ermC, ermT, erm45, msrA), sulfamethoxazole-trimethoprim (64.7/dfrA, dfrK), ciprofloxacin (45.9), tobramicin (38.8/ant4'), chloramphenicol (25.9/fexA, catPC221), gentamicin-tobramycin (25.9/aac6'-aph2"), clindamycin (11.8/lnuA, lnuB, salA), linezolid (4.8/cfr, optrA) and mupirocin (2.4/mupA). Most importantly, is the detection of cfr (in S. saprophyticus and S. epidermidis) and optrA (S. haemolyticus) among pigs and pig-farmers. Inter-pigs nasal carriage of S. sciuri, S. hyicus and S. haemolyticus with similar MDR genes were detected from the farms A-C. Also, similar MDR-S. saprophyticus isolates in a pig and pig-farmer from farm B were detected.

Conclusions:

The high-level of multidrug, within-host and intra-species resistome diversity in the nasal CoNS highlight their ability to be long-term AMR genes reservoir in healthy pigs and pig-farmers. Also, the detection of critical AMR strains carrying optrA- and cfr genes deserves comprehensive and continuous surveillance of MDR-CoNS at pig-farm levels.

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P02
Prevalence and antimicrobial resistance of *Enterococcus* spp. isolated from seafood samples

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Introduction:
Enterococci are ubiquitous bacteria that colonize the human and animal intestinal tract and are considered an opportunistic foodborne pathogen. The present study aimed to assess the prevalence and antimicrobial resistance of *Enterococcus* spp. strains isolated from retail seafood (fresh and frozen) purchased in local supermarkets in central Italy.

Methods:
Sampling was designed considering an expected prevalence of 20%, 95% confidence levels, 10% precision. Samples (n= 422) from seven seafood categories (fin and fish fillets, bivalve molluscs, crustaceans and cephalopods) were collected over one year and tested for the presence of *Enterococcus* spp. using selective culture. Isolates were identified using MALDI-TOF.

Results:
Overall, 288 (68%) samples resulted positive to enterococci. Cephalopods, salmons, bivalve molluscs and crustaceans showed a prevalence higher than 70%. An association between frozen *versus* fresh status was observed (OR = 1.69, CI95% 1.08-2.70, *p*= 0.0156). The predominant species were *Enterococcus faecalis* (48.6%) and *Enterococcus faecium* (17.4%). One hundred and two isolates were randomly selected and tested using minimal inhibitory concentration (MIC) test and interpreted according to the clinical breakpoints of the Clinical & Laboratory Standards Institute. Isolates showed a resistance to quinupristin/dalfopristin (52.9%), tigecycline (31.4%), tetracycline (27.5%), ciprofloxacin (18.6%), erythromycin (15.7%), and chloramphenicol (6.9%). None of the isolates was resistant to daptomycin. One isolate of *Enterococcus faecium* and one of *Enterococcus faecalis* were resistant to linezolid, whereas one *Enterococcus gallinarum* isolate showed an intermediate resistance to vancomycin.

Conclusions:
Here, we confirm that seafood can be a source of antibiotic resistant bacteria: hence, antibiotic resistance surveillance should include these commodities.

P03
Antimicrobial resistance and genomic characterization of *Salmonella enterica* strains isolated from chicken meat in Saudi Arabia

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Introduction: This study investigated genotypic and phenotypic antimicrobial resistance profiles, phylogenic relatedness, plasmid, and virulence composition of 39 *Salmonella Enterica* strains isolated from chicken meat samples using Whole genome sequencing (WGS) technology.

Methods*:* *Salmonella* isolates (n= 39) were identified through standard cultural and molecular detection methods. Antimicrobial susceptibility testing to different antibiotics was determined and genomic DNA was extracted. Libraries were constructed and sequenced using the Illumina MiSeq platform. Raw data were demultiplexed, trimmed, checked for quality, *de novo* assembled, and analyzed using appropriate bioinformatics tools.

Results: Four distinct serotypes were identified where *S.* Minnesota was the predominant serotype (41%), followed by *S.* Infantis (33.3%), *S.* Enteritidis (23.1%), and one isolate was detected for *S.* Kentucky (2.6%), with sequence types as followed: ST11, ST32, ST548, and ST198, respectively. Phenotypic resistance to tetracycline (91.2%), ampicillin (82.4%), sulfisoxazole (64.7%), and nalidixic acid (61.6%) was the most observed. Plasmidome showed the presence of eight incompatibility groups including Col, IncC, IncR, IncX, IncHI, IncFIB, IncFII, and IncI plasmids. Eleven *Salmonella* pathogenicity islands and up to 131 stress and/or virulence genes were identified. Phylogenetic analysis showed 4 phylogroups that were consistent with the identified ST profiles with high level of inter-diversity between isolates.

Conclusion: This is the first study that employs WGS for antimicrobial resistance characterization of *Salmonella* isolates from chicken meat samples in Saudi Arabia. Results derived from this study will be very helpful in future source-tracking especially during epidemiological surveillance and outbreak investigations associated with *Salmonella* foodborne illness.

P04
Are non-toxigenic *Clostridium difficile* strains reservoirs for antimicrobial resistance genes?

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Introduction: *Clostridioides difficile* is an important nosocomial pathogen. This study investigated the occurrence and characteristics of *C. difficile* in cats, dogs and their owners as its potential sources out of the hospital.

Methods: Rectal swabs and/or faeces were cultured on selective media. *C. difficile* isolates were characterized by PCR ribotyping and whole genome sequencing (WGS). Genetic relatedness was determined by core/whole genome multilocus sequence typing (wgMLST Bionumerics 8.1; cgMLSTFinder 1.2). Acquired resistance genes were detected using ResFinder 4.1. Antibiotic susceptibility to nine antimicrobials was determined using E-test.

Results: From 251 rectal swabs and/or faeces of dogs, cats and their owners, 37 isolates of *C. difficile* were cultured, 3 isolates were from humans. A total of 25 isolates (67.6%) carried toxin genes A and B, binary toxin genes were detected in 3 isolates (8.1%). The most prevalent ribotypes were 014 (n = 14, 37.8%) and 010 (n = 7, 18.9%). The results on antimicrobial resistance and detected molecular mechanisms are summarised in Table 1. Surprisingly, the expected phenotype was not observed for isolates with tetracycline efflux pumps. In addition, non-toxigenic isolates carried more antimicrobial resistance genes compared to toxigenic isolates ($p<0.001$).

Conclusions: The spectrum of ribotypes overlapped with published ribotypes from hospitalised patients. The non-toxigenic *C. difficile* strains could represent a hidden reservoir for resistance genes.

Number of isolates	Ribotype	Toxin genes	Clade	cgMLST	Antimicrobial resistance mechanisms		Resistance	Animal/Human
2	001-like	A, B	1	9227	sine		CIP (n = 1)	dog
1	009	tox-	1	1781	sine			dog
1	009-like	tox-	1	8846	sine		susceptible	dog
1	010	tox-	1	1025	*aph(3')-III, erm(B), tet(M), ant(6)-Ia*		CLI, ERY	dog
5	010	tox-	1	2422	*cfr(C), ant(6)-Ia*		CIP (n=1), CLI, ERY, LNZ	dog (n=4), cat (n=1)
1	010	tox-	1	20019	*tet(M), ant(6)-Ia*		CIP	human
6	014	A, B	1	512	sine		CIP (n=5)	cat
1	014	A,B	1	4616	sine		CLI, CIP	dog
1	014	A,B	1	6431	sine		CLI, CIP	dog
1	014	A,B	1	16765	sine		CIP	human
4	014	A, B	1	21746	sine		CIP	cat
2	014 (n=1), 449 (n=1)	A, B	1	2175	sine	Thr82Ile (GyrA)	CIP, MOX	dog
1	020	A,B	1	6091	sine		CIP	dog
2	020	A, B	1	6371	sine		CIP	dog (n=1), cat (n=1)
1	020	A, B	1	10818	sine		CIP	dog
1	023	A, B, Bin	3	1819	sine			human
3	039	tox-	1	15518	*aph(2'')-If (n=2), erm(B), tet(O), ant(6)-Ia*		CLI, ERY	dog (n=2), cat (n=1)
2	078-like	A, B, Bin	5	8969	aph(3')-III		susceptible	dog
1	449	A,B	1	6371	sine		susceptible	dog

Table1. A summary of *Clostridioides difficile* isolates characteristics.

Abbreviations: CIP = ciprofloxacin; CLI = clindamycin; ERY = erythromycin; LNZ = linezolid; MOX = moxifloxacin; cgMLST=whole genome multilocus sequence typing

Founding: This work was supported by IGA VETUNI 105/2021/FVL and National Institute for Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) - Funded by the European Union – Next Generation EU.

P05
Azole resistance in veterinary clinical *Aspergillus fumigatus* isolates from the Netherlands

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Introduction:

Aspergillus fumigatus (Af) is a saprophytic fungal pathogen causing opportunistic infections in animals and humans. Azole resistance has been reported globally in human *Af* isolates, but the resistance prevalence in isolates from animals is largely unknown.

Methods:

A retrospective surveillance study was performed in a collection of clinical *Af* isolates from various animal species collected in 2015-2020. Agar based azole resistance screening of all isolates was followed by *in vitro* antifungal susceptibility testing (AST) and *Cyp51A* gene sequencing of azole-resistant isolates.

Results:

Over the five year period 16 (11.2%) of 143 culture-positive animals harbored an azole-resistant *Af* isolate. Resistance frequencies varied from 0% (95% CI 0.0 – 15.5%) to 26.1% (95% CI 11.1 – 48.7%) per year without a clear trend. Resistant isolates were found in birds (15%; 2/13), cats (21%; 6/28), dogs (8%; 6/75) and free-ranging harbor porpoise (33%; 2/6). Azole-resistance was *Cyp51A* mediated in most isolates: 81.3% (T-67G/)TR34/L98H, 12.5% TR46/Y121F/T289A. In one azole-resistant *Af* isolate a combination of C(-70)T/F46Y/C(intron7)T/C(intron66)T/M172V/E427K single-nucleotide polymorphisms in the *CYP51A* gene was found. Of animals with an azole-resistant isolate and known azole exposure status 71.4% (10/14) were azole naive.

Conclusions:

Azole resistance frequency in *Af* isolates from animals is similar to that found in humans and was predominantly *Cyp51A* TR-mediated. Our data support the need for including veterinary isolates in resistance surveillance programs and azole resistance should be considered as a reason for therapy failure when treating aspergillosis in animals.

P06
Assessment of reliability and representativeness of antimicrobial susceptibility testing results for pathogens from dogs and cats in the Netherlands (2016 – 2020)

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Introduction
To promote prudent use of antimicrobials, it is extremely important to have access to representative and reliable antimicrobial susceptibility testing (AST) data. This study aimed to assess reliability and representativeness of available AST results of pathogens isolated from clinical samples from dogs and cats.

Methods
AST results and additional data (e.g., geographical location, infection site) were collected from 2016-2020 for *Escherichia coli* (ECO) and *Staphylococcus* (Staph) spp. from dogs and cats and *Pseudomonas aeruginosa* from dogs.

Results
Large numbers of isolates from dogs and cats were available and overall decreasing resistance percentages for all pathogens were observed during the study period. In both dogs and cats less than expected isolates originated from the province Noord-Brabant, while resistance percentages for multiple antimicrobials were higher in this province. The potential bias can be addressed by collecting more samples from Noord-Brabant or to correct for province in the analyses. Canine and feline ECO isolates originating from urine were overall more susceptible than ECO isolates from other infection sites. Whereas canine Staph isolates originating from skin showed higher resistance percentages for several antimicrobials than from other infection sites. Therefore, it might be better to evaluate and analyse resistance percentages stratified for infection site.

Conclusions
Overall, it can be concluded that the available AST data are fairly representative, however the data are characterised by some limitations which can lead to less representative and less reliable AST results. Correcting for most of these limitations in the analyses is feasible and should be taken into account in future studies.

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P07
Surveillance of antimicrobial resistance and genotypes of *Campylobacter jejuni* isolates in outpatients in Split-Dalmatia County, Croatia, 2021: the pilot study

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Introduction:

As the most reported zoonosis in humans globally accompanied by increasing antimicrobial resistance (AMR), campylobacteriosis is an important public health problem. The study aimed to analyze AMR data and resistance determinants in *Campylobacter jejuni* isolated from stool specimens of outpatients in Split-Dalmatia County (SDC), Croatia.

Methods:

C. jejuni strains were isolated and identified using standard bacteriological methods. Antimicrobial susceptibility testing was done according to EUCAST. MLST and WGS were used in resistome analysis

Results:

358 *C. jejuni* were isolated in outpatients in SDC in 2021. 268 (74.8%) isolates were resistant to ciprofloxacin, 76 (21.2 %) were tetracycline resistant, while resistance rates to erythromycin, gentamicin, and amoxicillin-clavulanate were low (< 5.6%).
We have also identified CIP_R/TC_R co-resistant *C. jejuni* strains as well as multiple resistant strains.
A small subset of 10 C*. jejuni* strains, isolated during the peak of infection in May and June were genotyped. They were genetically heterogeneous: 7 different MLSTs - ST were determined, and 7 MLST-CCs, while three strains were clonally related - they belong to MLST - ST 51 and MLST - CC 443 complex. Those strains showed ResFinder mutations: blaOXA-184 and 23S, cmeR, gyrAT86I aca. Additionally, they showed phenotype-genotype and spatiotemporal congruence

Conclusions:

AMR of C. *jejuni* strains in SDC to ciprofloxacin was high and moderate to tetracycline. CIP_R/TC_R co-resistant and multiple resistant strains were also identified.
Although WGS analysis was made on a small sample of *C. jejuni* strains that was genetically heterogeneous, clonally related strains have also been found.

P08

Study on *Pseudomonas aeruginosa* strains isolated from animals in a One-Health perspective

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Introduction:

Surveillance of animal-human cross-exchange of bacteria and mobile genetic elements is a specific objective of the one-health strategy to curb AMR. This study was due to explore whether animals host *Pseudomonas aeruginosa* (PA) clones of importance in human infections.

Methods:

To avoid over-representation, specific epidemiologic requirements (cross-checked by a decision-making algorithm linked to the LIMS) were used to sample PA isolates detected while performing some 89,000 bacteriological tests during 2018 - 2020 at IZSVe (North-East Italy). PA isolates originated from food-producing animals (92%), pets (7%) and wildlife (1.5%).

A subset of PA isolates was subtyped by MLST (online database https://pubmlst.org/), and presence of *exoS*, *exoT*, *exoU* and *exoY* assessed by PCR. MICs of 11 antibiotics of interest for human therapy were tested and interpreted according to human clinical breakpoints.

Results:

Fifty out of 232 strains collected were fully characterized. Among 46 different STs detected 7 were of human interest, including ST111 and ST395 high risk epidemic clones (Petitjean et al., 2017) detected in cattle and chicken samples. Forty-five isolates (90%) carried at least three genes coding for exotoxins.

All but three isolates were susceptible to 11 antimicrobials tested. Three isolates resistant to fluoroquinolones included a ST17 isolate from a dog (table 1).

Conclusion:

PA isolates from our sampled animals displayed high genetic diversity and low levels of resistance against antimicrobials of relevance for human therapy. ST17 clones were described in human samples, thus detection of a ST17 levofloxacin resistant isolate from a dog shows the exchange possibility between human and companion animals.

Table 1. MIC distribution of 50 strains of *Pseudomonas aeruginosa*. White boxes indicate the antibiotic concentration range tested. Human clinical BPs (HBPs) are shown (EUCAST). “NA” means that HBPs are not available.

	0,25	0,5	1	2	4	8	16	32	64	128	HBP
Amikacin					50						>16
Piperacillin/tazobactam constant 4					48		2				>16
Levofloxacin		46		1	2	1					>2
Nitrofurantoin										50	NA
Doripenem	50										>2
Imipenem	47	1	2								>4
Meropenem					8						>8*
Gentamicin			40		8	2					NA
Ceftazidime		8	1	33	8						>8
Cefepime				48		2					>8
Ciprofloxacin	47		1	2							>0,5

***Indications other than meningitis

P09
Genomic diversity and antimicrobial resistance of *Klebsiella pneumoniae* strains detected in Central Italy inside and outside the clinical setting

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Introduction:

Klebsiella pneumoniae (*Kp*) strains resistant to critically important antimicrobials (CIA) pose substantial challenges for treatment of hospital and community-acquired infections (1). Origin of the strains could pose interesting questions about antimicrobial resistance (AMR). The aim of this study was to investigate the *Kp* population using whole genome sequencing (WGS) inside and outside clinical settings.

Methods:

We tested 330 *Kp* strains from foods, environment, feed, animals and humans, collected in Central Italy between 2018-2022, for antimicrobial susceptibility to carbapenems, 3rd and 4th generation cephalosporines and fluoroquinolones (2).
WGS data analysis was carried out using an in-house pipeline (3). Sequence types (ST) and AMR genes were determined using MLST scheme (4) and ResFinder database (5) respectively.

Results:

According to MLST, 149 ST were identified with ST512 (7.7%) and ST307 (5.4%) mainly detected in clinical settings, meanwhile ST35 (4.8%), ST45 (4.5%) and ST37 (1.2%), even if of clinical origin were found also outside. Seventy *Kp* strains were carbapenem resistant, and 60 of them harbored KPC-3 genes. Eighty strains were phenotypically resistant to cephalosporins, and 2 isolates did not carry any genetic determinants. Finally, 68 were phenotipically resistant to fluoroquinolones, but 88 *Kp* harbored resistance genes (Figure 1).

Conclusions:

Heterogeneous population was revealed in non-clinical settings but high-risk and CIA resistant clones were detected mostly in the clinical ones. Based on these findings, the presence and spread of resistant strains in clinical settings should be the objective of a on purpose plan, but the transmission dynamics with outside strains should be investigated.

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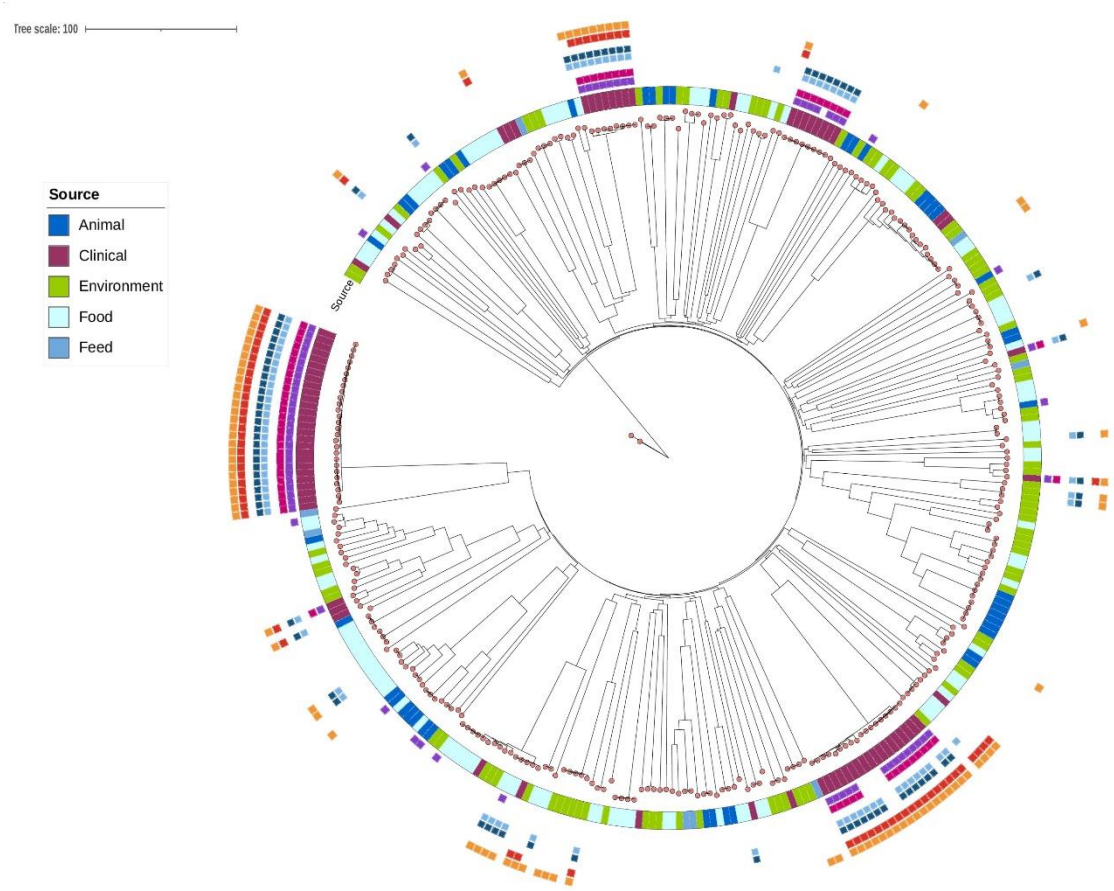
Figure 1 Caption:

Phylogenomic reconstruction based on *Klebsiella pneumoniae* cgMLST profiles. The tree was annotated using as the first layer the source origin. Phenotypic anitimicrobial resistance (AMR) and genotypic AMR features for each class are reported in the following order: carbapenem phenotypic resistance (purple) and resistance genes (magenta); 3rd and 4th generation cephalosporines phenotypic resistance (light blue) and resistance genes (blue); fluoroquinolones phenotypic resistance (red) and resistance genomic determinants (orange).



P10
Tracing the spread of the emerging multidrug resistant *Salmonella* Muenchen in the poultry production system in Israel

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Introduction:

Foodborne salmonellosis, a zoonotic infection associated with poultry, poses a global threat to public health. Since 2017, the identification of *Salmonella* Muenchen in human clinical cases, poultry and food sources in Israel has increased dramatically. Controlling infection in poultry is essential for mitigating the spread of this emerging multidrug resistant pathogen. However, to design effective control strategies, the direction of transmission to broiler flocks (from the breeder parents vs other broiler farms) and the presence of antimicrobial resistance determinants in strains from all production stages needs to be ascertained. We aimed to trace the direction of transmission and to characterize antimicrobial resistance in different production stages of poultry

Methods:

We collected samples from 43 and 35 broiler farms originating from 13 and 9 heavy breeders farms positive to *S*. Muenchen and negative to *Salmonella*, respectively.

Results:

Broiler farms were found positive to *S.* Muenchen regardless of the status of the parent heavy breeder farm (26/43 (60%) and 22/35 (63%), respectively). Whole genome sequence analyses of 60 strains also demonstrated a lack of clustering between broiler and their parent heavy breeder farm. In addition, *qnr* genes, conferring resistance to quinolones, were found in 58% of the sequences from all production stages.

Conclusions:

Our findings support that horizontal transmission between broiler farms plays a critical role in *S*. Muenchen spread. Therefore, the common approach of vaccinating heavy breeders may not suffice as a control strategy. Moreover, the high prevalence of quinolone resistance determinants may suggest a potential selection force for *S.* Muenchen propagation on farms.

P11
One Health monitoring of ESBL/AmpC-producing *Escherichia coli* in Northern Italy

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Introduction:

Effective antimicrobial resistance (AMR) surveillance cannot be achieved without a One Health approach. *Escherichia coli* producing extended-spectrum β-lactamases (ESBLs) and other β-lactamases (AmpCs) are a major AMR issue in human and veterinary medicine. Here we present preliminary regional-scale data regarding the occurrence of *E. coli* producing ESBL/AmpC in samples of human, animal and environmental origin in Northern Italy.

Methods:

A total of 1093 samples, including human wastewater (n=342), livestock manure (n=126), fresh water (n=163), faeces of wild birds (n=342) and wild mammals (n=200), were collected from June 2021 to December 2022 in mountain and lowland areas of Lombardy region, Northern Italy. The presence of ESBL/AmpC-producing *E. coli* was investigated through phenotypical and molecular methods.

Results:

Overall, 32.2% of the analysed samples were positive for ESBL/AmpC-producing *E. coli*. The probability of isolating a resistant strain strongly depended on the sample type ($p<0.0001$): prevalence of resistant *E. coli* in livestock manure and human wastewater was around 60%, whereas fresh water and wildlife showed prevalences lower than 15% (Figure 1). A higher prevalence of resistant strains was recorded in bovine (73.2%) and swine (76.9%) manure compared to poultry (42.1%) ($p=0.0007$). The most frequently detected ESBL/AmpC genes were $bla_{CTX\text{-}M}$ (76.4%) and bla_{TEM} (47.7%), followed by bla_{CMY} (4.5%) and bla_{SHV} (4.0%).

Conclusions:

Although ESBL/AmpC-producing *E. coli* were more common in human and livestock samples, they were also found in fresh water and wildlife, where there should be no exposure to antimicrobials. These results confirm the importance of One Health surveillance.

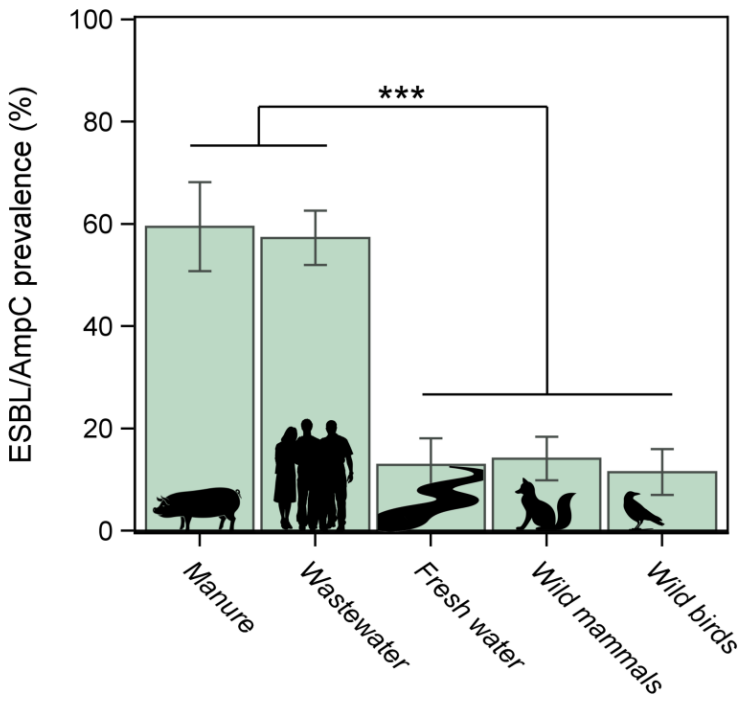

Figure 1. Prevalence of ESBL/AmpC-producing *Escherichia coli* isolated in samples of different origin (n=1093) in Northern Italy. Logistic regression analysis showed that human and livestock waste had a higher probability of harbouring resistant strains compared to environmental samples ($p<0.0001$). Error bars indicate 95% confidence limits.

P12
Assessment of representativeness and reliability of antimicrobial sensitivity testing results of *Salmonella* Dublin *and Salmonella* Typhimurium from veal calves in the Netherlands from 2016 – 2020

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Introduction:

Further promotion of prudent use of antimicrobials necessitates access to representative and reliable antimicrobial susceptibility testing (AST) data. This study aimed to assess representativeness and reliability of AST results available at Royal GD for *Salmonella* Dublin (SDU) and *Salmonella* Typhimurium (STY) from clinical samples from Dutch veal calves.

Methods:

AST results and additional data (e.g., location of the farm and the associated veterinary practice) were collected from 2016-2020 and statistically evaluated.

Results:

After validation of the data, 202 unique SDU and 316 STY isolates remained available for analyses. These relatively low numbers made it difficult to study the association between AMR results and different parameters. Also, the AMR percentages were less precise, especially when results of only one year were presented, with 95% confidence intervals ranging up to 30%. Results showed a positive trend for antimicrobial susceptibility of SDU and STY from 2016 to 2020, with decreasing odds for resistance for the majority of included antimicrobials.
The distribution of SDU and STY isolates across the Netherlands was similar to the population density of veal calves and the individual provinces did not have significant different odds for isolates being resistant. Furthermore, isolates originating from farms associated to the same veterinary practice were more alike than isolates originating from farms associated to different veterinary practices.

Conclusions:

The low number of isolates does lead to less *representative* and *reliable* AST results. Therefore, effort should be made to increase the number of isolates from veal calves.

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P13
Science to policy translation in One Health European Joint Programme (One Health EJP): Building collaboration, communication and trust

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Introduction:

The One Health European Joint Programme (One Health EJP) is a multidisciplinary initiative that has successfully brought together 44 partners across Europe to address foodborne zoonoses, AMR, and emerging infectious threats. Among its 30 joint projects, seven (ARDIG, FARMED, FED-AMR, FULL-FORCE, IMPART, RaDAR and WORLDCOM) focused specifically on AMR.

Methods:

The dissemination of the gap-driven research results is supported by a dedicated work package on Science to Policy Translation, in collaboration with a Communications Team, and there is close dialogue with European and global stakeholders (ECDC, EFSA, EEA, EMA, FAO, WOAH, WHO-Europe) to achieve maximum impact of outcomes.

Results:

In time, the collaboration with the stakeholders mediated by the Science to Policy Translation team has strengthened trust between the many actors, which is crucial for the transfer of knowledge from science into policy. Uptake and application of the solutions developed by One Health EJP, advocacy of One Health approach, and preparation for future One Health collaborations, are evidence of the impact of the work.

Conclusions:

The One Health collaborations and the trust built will continue beyond the lifetime of the programme. The solutions produced during the One Health EJP will continue to contribute to prevention, preparedness and response to One Health issues – including AMR – in Europe and globally.

P14
Characterisation of ESBL producers from aquatic invertebrates by whole genome sequencing: the role of rivers in cross-border spread

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Introduction:

The One Health concept identifies water bodies as reservoirs of multiresistant bacteria.

Methods:

This study focused on aquatic invertebrates collected from the River Berettyó two kilometers downstream of its entry from Romania to Hungary (47.25276, 21.83794). Fifty banded demoiselle (*Calopteryx splendens*) larvae were collected, killed and cultured in Mueller-Hinton broth then on eosin methylene blue agar both supplemented with 2 mg/l cefotaxime. Recovered 3rd generation cephalosporin resistant isolates were sequenced using an Oxford Nanopore MinIOn platform, then the replicons, resistome, MLST and cgMLST type were determined after *de novo* assembly.

Results:

The prevalence was 19/50 (38%); three Citrobacter freundii, nine Escherichia coli, five Klebsiella pneumoniae, three Morganella morganii and one Raoultella planticola were isolated. Of the K. pneumoniae sequenced successfully one ST147 carrying blaCTX-M15 and QnrB17, and three ST1119 carrying blaCTX-M15 and aac(3)-IIe were identified. Among E. coli isolates one ST131 (cgMLST179733) carrying blaCTX-M15; one further ST131 (cgMLST21405) carrying blaCTX-M15 and blaCTX-M27; one ST168 (cgMLST81383) carrying blaCTX-M27 and blaCMY-59 on the same plasmid, two ST182 (both cgMLST148002) carrying blaCTX-M3 and blaCTX-M15; two ST609 (both cgMLST180278) carrying blaCTX-M65 (the gene identified first in Hungary) and qnrS1; one ST744 (cgMLST96978) carrying blaCTX-M3 and blaCTX-M55 on two different plasmids as well as three isolates of unidentified STs.

Conclusions:

High prevalence of MDR bacteria including strains of potential human (ST1119 *K. pneumoniae*, ST131 *E. coli*) and veterinary (ST744 *E. coli*) interest points to the role of rivers and invertebrates inhabiting them as reservoirs and vehicles of transboundary spread of multiresistance as highlighted in the One Health concept.

P16
Multiresistant Enterobacterales in long-distance migrant Common tern (Sterna hirundo) chicks

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Introduction:

According to the One Health concept environment and wildlife may serve as reservoir for antimicrobial resistance. Our group investigates the prevalence of multiresistant Enterobacterales in mixed waterbird colonies, the present study concentrates on the long-distance migrant species Common tern (*Sterna hirundo*).

Methods:

We collected fecal samples from 84 chicks in three colonies between 2020-22. Samples were cultured on eosin-methylene-blue agar supplemented with 2 mg/l cefotaxime. Sixteen chicks were positive (19%), 18 *E. coli*, 1 *E. marmotae* and 1 *E. alberti* were found and their whole genomes were sequenced on Oxford Nanopore MinION platform.

Results:

We found *E. coli* sequence types with human and avian pathogenic importance, ST155 (two cgST136648, carrying $bla_{CTX\text{-}M\text{-}1}$ and one cg165923, carrying $bla_{CMY\text{-}59}$), ST648 (one cgST147656; carrying $bla_{CTX\text{-}M\text{-}}$14) and ST23 (one cgST34315; carrying $bla_{CTX\text{-}M\text{-}1}$). We identified STs that occur frequently in human and production animal samples, ST58 (one cgST9013, carrying $bla_{CTX\text{-}M\text{-}65}$; and one cgST175317, carrying $bla_{CTX\text{-}M\text{-}15}$), ST1718 (one cgST143413 and one cgST23658; both carrying $bla_{CMY\text{-}59}$) and ST744 (one cgST86922, carrying $bla_{CTX\text{-}M\text{-}15}$).

Conclusions:

Terns breed in mixed colonies with gulls feeding on human waste, which may be the source of these bacteria. On the other hand, terns may connect distant places by the global network of migration routes, which may contribute to the spread of antimicrobial resistance.

P17
Implication for human clinical settings of a restrictive intervention on 3rd generation cephalosporin (3GC) use in dairy farms

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3GCs are important therapeutics for humans and also prescribed for animals. In June 2018, restrictions were introduced on UK dairy farms almost eliminating their use. We hypothesised that this would cause a reduction in CTX-M-mediated but not AmpC-mediated 3GC resistance (3GC-R), because the latter also confers resistance to amoxicillin-clavulanate, where usage was not restricted.

Methods:

828 faecal samples were collected on 20 South-West English dairy farms in 2017/18 (Pre-intervention) and 645 samples in 2020/21 (Post-intervention). 3GC-R *E. coli* where isolated by selective plating. 3GC-R *E. coli* were isolated from human bloodstream infections in the same geographical area in 2017-18 (n=68) and 2020 (n=67). Whole genome sequencing (WGS) was used to identify 3GC-R mechanisms.

Results:

Pre-intervention, the ratio of CTX-M to AmpC mediated 3GC-R in farm *E. coli* was 50:50; post-intervention it had shifted to 37:74 in favour of AmpC, as predicted. In contrast, the ratio was 90:10 in favour of CTX-M in human bloodstream isolates. Notably, while bla_{OXA-1} (an alternative cause of amoxicillin-clavulanate resistance) was never associated with bla_{CTX-M} in 3GC-R farm isolates, 56% of bla_{CTX-M} positive human isolates also carried bla_{OXA-1}.

Conclusion:

Because CTX-M-positive 3GC-R farm isolates are rarely amoxicillin-clavulanate resistant, reducing 3GC use whilst maintaining amoxicillin-clavulanate use has shifted 3GC-R towards an AmpC-mediated mechanism, which is mutational, and non-transmissible. However, this might not translate to human settings because a majority of CTX-M-positive isolates are 3GC/amoxicillin-clavulanate dual resistant due to CTX-M/OXA-1 linkage. Hence reductions in 3GC and amoxicillin-clavulanate may be necessary to reduce the burden of CTX-M-positive *E. coli*.

P18
Establishing a One Health Antimicrobial Resistance Community of Practice: The UK FAO Reference Centre for AMR Experience

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Introduction:
The UK FAO Reference Centre for Antimicrobial Resistance has established a One Health AMR Community of Practice (CoP) to support the FAO's global effort to combat AMR across sectors in agrifood systems.

Methods:
The CoP brings together current and prospective collaborators and other groups or individuals who are involved and interested in topics related to AMR. Its main purpose is to strengthen the understanding and cooperation among participants in dealing with issues related to AMR, thereby supporting the One Health approach in tackling this challenge. Activities are aimed at increasing awareness and engagement among our partners and other key stakeholders, and to assist in improving laboratory capacity and promote good laboratory practices among participants.

Results:
Membership comprises 80+ AMR experts representing 22 countries. We meet virtually once every three months to share expertise and build professional networks through presentations from AMR experts, interactive learning sessions, and professional development. A popular component of our CoP is a regular session called "Meet Your Community" in which members present on their institutions and the AMR related work they are currently doing, providing opportunities to share new ideas and to effectively network and collaborate among the participants.

Conclusions:
Establishing a virtual One Health AMR CoP has provided an effective platform to continually engage people from varied sectors in developing the CoP members' capabilities in addressing AMR. It is our long-term goal that the activities of this CoP will contribute to overall global efforts in tackling AMR across One Health sectors.

P19

Dissemination of *Streptococcus suis* serotype 9 strains characterized by a reduced susceptibility to beta-lactams in Italian pig farms

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Introduction:
Streptococcus suis is one of the most important swine pathogens and an emerging zoonotic agent. In a previous study we described an increase of *S. suis* serotype 9 (SS9) infections in pig herds in Italy: most isolates belonged to sequence-type (ST) 123, and were characterized by a reduced sensitivity to penicillin. The aim of this work was to investigate the trend of sensitivity to beta-lactams of SS9 in Italy from 2002 to 2021.

Methods:
We analysed 66 isolates of SS9 isolated from clinical cases of streptococcosis, in terms of ST, susceptibility to antibiotics, presence of genes coding for antibiotic resistance and virulence and substitutions in the Penicillin Binding Protein (PBP). The genomes of the Italian isolates were compared with the SS9 genomes available in public repositories.

Results:
The most frequent STs were ST123 and ST16, which differed in terms of virulence factors and sensitivity to beta-lactams. The isolates belonging to ST123, ST1954 and ST94 clustered together in the same phylogenetic group with isolates from Spain, and distinct from isolates of other origin. A reduced susceptibility was observed not only to penicillin, but also to ceftiofur and ampicillin, without reaching clinical resistance. The susceptibility to penicillin decreased over the observation period, and this reduction was associated with ST123, ST1540 and ST1953. Strains characterized by low susceptibility to beta-lactams showed a characteristic mosaic structure of the PBPs.

Conclusions:
In conclusion, the susceptibility to penicillin of SS9 has been progressively decreasing in Italian herds in the last twenty years, posing a threat to public health.

P20
Fluoroquinolone and extended spectrum cephalosporin resistance in commensal *Escherichia coli* on Irish pig farms

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Introduction:

Antimicrobial resistance (AMR) to the Highest Priority Critically Important Antimicrobials (HP CIA) such as fluoroquinolones (FLQ) and third and higher generation cephalosporins (ESC) is a major public health issue. Pigs are a reservoir for FLQ- and ESC-resistant *Escherichia coli*. The objective of this study was to investigate the evolution of FLQ and ESC resistance in *E. coli* throughout the pig production cycle on Irish pig farms.

Methods:

Twelve Irish farrow-to-finish farms participated in a longitudinal study. Ten litters of piglets were selected from each farm; pooled faecal samples were collected during each production stage (piglet, 1st stage weaner, 2nd stage weaner and finisher) and from the piglet's dams. FLQ- and ESC-resistant *E. coli* were selected using media supplemented with 1 mg/L ciprofloxacin and 1 mg/L cefotaxime respectively and plate counts were used to calculate the proportions of resistance.

Results:

All farms were positive for FLQ-resistance at least once during the study while two farms were negative for ESC-resistance throughout. Proportions of resistance to both antimicrobials were generally highest in piglets (FLQ: median = 0.03, range = 0-0.9; ESC: median = 0.00005, range = 0-0.15) and lowest in finishers (FLQ: median = 0, range = 0-0.14; ESC: median = 0, range = 0-0.01). Proportions of resistance to both antimicrobials in piglets and sows were significantly correlated (Spearman $\rho = 0.8$, $p \leq 0.002$ for both).

Conclusions:

This study identified the farrowing house as a hotspot for resistance to HP CIAs on Irish pig farms.

P21
Antimicrobial resistance in commensal *Escherichia coli* on Irish pig farms: a longitudinal study

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Introduction:

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

Methods:

Twelve Irish farrow-to-finish farms were assigned to three groups according to their level of AMU in medicated feed (low, moderate, high). Ten litters of piglets were selected from each farm; pooled faecal samples were collected during each production stage (piglet, weaner, grower and finisher). Twenty *E. coli* isolates from each sample were tested against a panel of 16 antimicrobials. Mixed effects logistic regression models of resistance to each antimicrobial were constructed using AMU practice and stage of production as explanatory variables.

Results:

The highest frequencies of resistance were to doxycycline, ampicillin, trimethoprim/sulfamethoxazole, gentamicin and the fluoroquinolones. Resistance to these agents was highest in 1st stage weaners except for fluoroquinolones where it was highest in piglets. Resistance was lower on farms not using medicated feed (low AMU) except for fluoroquinolone and trimethoprim/sulfamethoxazole resistance. Resistance to amikacin or imipenem was not observed and resistance to the cephalosporins was low.

Conclusions:

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

P22
The evolution of the faecal resistome is shaped by age and antimicrobial use during the pig lifecycle

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Introduction:

Although new European Union regulations aim to restrict antimicrobial use (AMU) in livestock, antimicrobials remain an essential tool in ensuring animal health and welfare. Therefore, studies investigating how different levels of AMU affect the evolution of antimicrobial resistance during the production cycle are required. This study investigated the dynamics of the porcine faecal resistome in batches of pigs on Irish farms with differing levels of AMU throughout their entire lifecycle.

Methods:

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

Results:

Genes conferring resistance to tetracyclines, macrolide, aminoglycoside and beta-lactams were the most abundant antimicrobial resistance genes (ARG) on all farms. The abundance of ARGs in growing pigs peaked during the weaner stage decreasing thereafter while the lowest abundance was observed in sows. Abundance was lower on low AMU farms compared to moderate or high AMU farms. The composition of the resistome was strongly influenced by the composition of the microbiota in an age dependent manner.

Conclusions:

This is the first multi-farm longitudinal study to investigate the dynamics and evolution of the faecal resistome throughout the pig production cycle and gives important insight into how age and antimicrobial use impact the resistome in pig farming.

P23
Application of Culturomics and Whole Genome Sequencing for the detection of antibiotic resistance genes in novel bacteria isolated from Wastewater Treatment sites.

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Introduction:

Wastewater Treatment sites (WWTs) are significant reservoirs for Antibiotic Resistance Genes. Very few culture-based studies have worked on the microbial ecology of WWTs and the dispersal of ARGs in nature. Unknown and uncultured microorganisms may contribute to the ARGs spread. We implemented «culturomics» to isolate unknown microorganisms and decipher the bacterial ecology of raw efflux WWTs samples. We further aim to perform Whole Genome Sequencing to identify and type the novel bacteria and search for ARGs on their genome.

Methods:

Raw untreated samples from the WWT of Heraklion, Crete (Greece) are collected for the needs of SARS-CoV-2 surveillance. The samples are cultured for up to 30 days in 25 conditions and 15 solid media. Colonies are initially identified by MALDI TOF-MS. Unidentified bacteria are subjected to 16s rRNA sequencing. Bacteria whose 16s reveals similarity >97% to already published sequences, are characterized by WGS. Bioinformatics is used to detect and compare ARGs of commensal and pathogenic bacteria of public health interest kept at our bank.

Results:

We have cultured 15 raw samples, generating 300 species (Figure 1) and 5.000 isolates. The most represented taxa are Enterobacteriaceae followed by Enterococcaceae.16s rRNA sequencing has confirmed so far the isolation of at least seven (7) novel and 14 previously uncultured bacteria. The search of ARGs in the new microorganisms will follow.

Conclusions: Culturomics, a novel scientific field, can become a useful tool in understanding the global resistome by unraveling the role of previously uncultured bacteria in the AR spread.

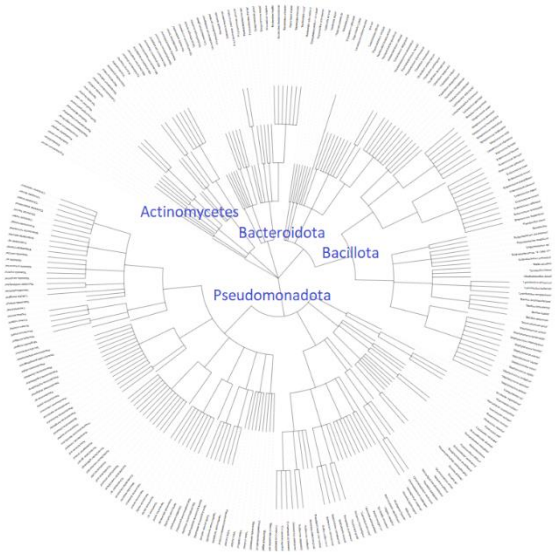

Figure 1: Phylogenetic tree (Generated by iTOL, https://itol.embl.de/)

P24
Loop-mediated isothermal amplification method as a point-of-care test for ETEC identification

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Introduction:

Post-weaning diarrhea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC) remains a major concern for the pig industry. Here, we developed a point-of-care test for direct detection of ETEC and resistance to first-line antimicrobials used to treat PWD, namely neomycin and tetracycline.

Methods:

The test uses colorimetric loop-mediated isothermal amplification (LAMP), and primers were designed based on the predominant ETEC fimbrial and toxin genes and genes conferring resistance to neomycin and tetracycline. **The performance of the LAMP test was** evaluated using 442 *E. coli* isolates from Danish pig farms, and PCR results and antimicrobial susceptibility testing were used as the reference standards.

Results:

The LAMP test for ETEC detection was in full agreement with PCR results when compared to susceptibility results. The sensitivity and specificity for ETEC detection were 92% and 99%, respectively, whereas the corresponding values for resistance identification were 91% and 88% for neomycin; 84% and 88% for tetracycline, respectively. The detection limit for ETEC genes in a fecal suspension was $1x10^3$ CFU/ml. However, the LAMP test was less effective when tested directly on nine ETEC-positive clinical specimens, with sensitivity and specificity for ETEC detection at 69.2% and 62.5%, respectively.

Conclusions:

We propose a simple, rapid, and cost-effective point-of-care test that has the potential to be used for rapid on-site ETEC detection. Further optimization of the test may improve its performance on clinical samples.

P25
Wild animals as sentinels of antimicrobial resistance of *Salmonella* spp.

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Introduction:

Antibiotic resistance in *Salmonella* species, frequent etiologic pathogens of foodborne disease in humans, is a serious health problem worldwide. *Salmonella* is a zoonotic agent and wild animals might represent a risk for *Salmonella* spp. transmission to humans. Wild species are an important reservoir of antimicrobial-resistant bacteria and could be used as sentinels for surveillance. The aim of the study was to evaluate antimicrobial resistance of strains isolated from wild animals during 2017-2021 in northwest Italy.

Methods:

From a total of 289 wild animals (134 wild boars, 66 birds, 33 reptiles, 22 foxes, 34 other species), 289 strains were identified and disk diffusion test was performed to detect resistance up to 13 antimicrobials, belonging to 10 different classes. Strains displaying intermediate susceptibility were considered resistant.

Results:

The results showed that 28.4% of the strains were resistant to at least one antimicrobial agent while 71.6% were susceptible to all drugs tested. In particular, 22.7% of the strains showed resistance to tetracycline, 17.8% to sulfamethoxazole and 12.5% to ampicillin. Multidrug resistant patterns (resistance to three or more antimicrobial classes) are rather frequent, accounting for 34.1% of the resistant strains. Regarding Highest Priority Critically Important Antimicrobials, 9.3% of the strains were resistant to quinolones, 7.3% to cefotaxime and 2.8% to ceftazidime.

Conclusions:

Our study showed the circulation of resistant *Salmonella* spp. in wild animals living in this area of Italy, highlighting the possible role of these animals as reservoir and spreaders of antimicrobial resistant pathogen strains.
This study was funded by Italian Ministry of Health (IZSPLV11/19RC).

P26
Consensus amongst human and animal health experts on the use of high importance-rated antimicrobials in animals – a delphi study

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Introduction:
In Australia, high importance antimicrobials are those that are, “essential for the treatment or prevention of infections in humans where there are few or no treatment alternatives for infections”. This study describes a delphi study to achieve consensus on the circumstances under which antimicrobials with high importance to human health can be used in animals.

Methods:
The delphi consensus method is a group facilitation process that provides guidance around issues where higher quality evidence may not be available or evidence may be contradictory. 44 human and animal health experts including veterinarians, physicians, and microbiologists undertook three rounds of anonymous online surveys.

Results:
Consensus was defined as 80% or more of respondents selecting the same option for a question and was achieved on 6 items.
Consensus items:
1.The country-specific rating system should take precedence over any other rating system
2.International prescribing guidelines should be adapted to account for the country-specific rating system.
3.Veterinarians should be able to create local practice-specific antimicrobial use protocols but should not create their own importance rating systems.
4.Use of high importance antimicrobials is allowed after antimicrobial culture and sensitivity testing confirms resistance to low and medium rated antimicrobials
5.The use of high importance antimicrobials in veterinary medicine should NOT be banned.
6.Any use of high importance antimicrobials not registered for use in animals must be reported to a central authority.

Conclusions:
The delphi process was valuable in facilitating consensus amongst experts from a broad range of health backgrounds and experience.

P27
Towards harmonized methodologies in veterinary clinical bacteriology– outcomes of a European survey

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Introduction: Veterinary microbiological diagnostic laboratories (VMDLs) play a key role in antimicrobial stewardship by providing guidance for antimicrobial treatment and by contributing to AMR surveillance.
Methods: The European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT) has designed and distributed a survey aiming to map diagnostic methodologies across VMDLs in 34 European countries. The survey focused on practices and interpretive criteria used for bacteriological culture and identification (C&ID), and antimicrobial susceptibility testing (AST) of animal bacterial pathogens.
Results: A total of 290 laboratories responded, representing a mixture of academic (39%), governmental (33%), and private (28%) laboratories. Average C&ID turnaround varied from 1-2 days (78%) to 3-5 days (20%), and 6-8 days (0.5%) with similar timeframes for AST. Biochemical ID systems (e.g., API kits) were the most used (56%), followed by MALDI-TOF MS (46%). For AST, Kirby-Bauer disc-diffusion (DD) and MIC determination were conducted by 44% and 33% of laboratories, respectively. A combination of EUCAST and CLSI clinical breakpoints (CBPs) was the most common approach for interpretation of both DD (41%) and MIC (47%), whilst in some countries (i.e., France) national guidelines were used. Fifty three percent of laboratories used human CBPs for AST interpretation when veterinary breakpoints are lacking. Furthermore, 48% and 46% of VMDLs routinely screened isolates for methicillin resistance and ESBL production, respectively.
Conclusions: A broad variety of methodologies were identified for C&ID and AST in European VMDLs, which emphasizes the need to harmonise diagnostic methodologies to benefit rational antimicrobial use and ultimately improve animal and public health

P28
Assessment of representativeness and reliability of antimicrobial sensitivity testing results of *Actinobacillus pleuropneumoniae*, *Escherichia coli* and *Streptococcus suis* from pigs in the Netherlands from 2016 – 2020

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Introduction:

Access to representative, reliable antimicrobial susceptibility testing (AST) data is a prerequisite for further promotion of prudent use of antimicrobials and provision of solid evidence for policies aimed at reducing antimicrobial resistance levels in animal bacteria. Therefore, this study (financed by ZonMw) aimed to assess representativeness and reliability of at Royal GD available AST results of *Actinobacillus pleuropneumoniae* (APP), *Escherichia coli* (ECO), and *Streptococcus suis* (SSU) from pigs.

Methods:

AST results (Minimal Inhibitory Concentration (MIC)) and additional data (age category, farm and province of origin) were collected from the period 2016-2020 and statistically evaluated.

Results:

Overall, the number of APP, ECO and SSU isolates were well representative considering the number of pigs (and number of farms) per province they originated from. Although less APP isolates were available compared to ECO and SSU, for all bacterial species fairly precise estimations of resistance levels were determined as shown by the confidence intervals in the MIC distributions (<17% but most <9%). The dataset also allows for detection of year-to-year trends in resistance; hardly any significant changes in resistance percentages in time were found. Multilevel analyses revealed significant associations between resistance levels and age category (ECO and SSU); generally, resistance levels decreased with increasing age. For several antimicrobials a significant association between farm of origin and level of resistance was shown.

Conclusions:

All factors associated with susceptibility levels should be considered before sharing aggregated susceptibility patterns for use in antimicrobial treatment guidelines and in veterinary practice.

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P30
Use of essential oils in the fight against antibiotic resistance from aquacultures

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Introduction:
Aquaculture is one of the most promising food industries with drastically increasing *pro capite* fish/shellfish consumption. Intensive techniques and the growing use of sub-therapeutic doses of antibiotics, increase both the selective pressure on resistant bacteria and the transfer of mobile resistance genes into both the aquatic and terrestrial environment. Unfortunately, little has been done to identify alternative antimicrobial natural approaches in fish farms.
The aim of this research is to identify the effectiveness and the safety of both essential oils (EOs) and commercial formulations based on EOs (CF-EOs) to control the fishes' pathogenic bacteria growth and the development of their biofilms in aquacultures.

Methods:
The antibacterial efficacy of eleven EOs and two CF-EOs was tested against nine ATCC and seven bacteria belonging to fish farms. Chemical analysis of both EOs and CF-EOs was achieved by GC-MS analysis. MIC and MBC values were obtained using broth micro-dilution tests according to EUCAST guidelines. The efficacy on biofilm disruption was evaluated with both conventional *in vitro* investigations and live and dead staining performed on biofilms grown directly on crustacean carapace. Finally, *in vivo* models of zebrafish embryos were used to evaluate the safety of the most active EOs and CF-EOs.

Results:
The data show that concentrations lower than 0.05% v/v of EO and CF-EO, properly delivered, are safe and able to modulate the growth of pathogenic bacterial strains and their biofilms.

Conclusion:
This paves the way for green treatments in the one health prevention of fish epidemics.

Minimum inhibitory concentration							
Bacteria	*Yersinia ruckeri*	*Vibrio harveyi*	*Photobacterium damselae subsp. piscicida*	*Pseudomonas aeruginosa*	*Aeromonas salmonicida subsp. salmonicida*	*Pseudomonas anguillis eptica*	*Tenacibaculum maritimum*
Strain	ITT 100/16	ITT 11/16	ITT 210 B/21	ATCC 27853	ATCC 33658	180/16/ B	ITT 314/17/A
Oil blend gl	0,585 ±0,275	1,040± 0,450	0,195±0	>3,125	0,098± 0	0,024±0	0.006±0
Oil bland br	0,195± 0	0,130± 0,056	0,024±0	>3,125	0,104± 0,117	0,001±0	0.006±0
Origanum vulgare	0,050± 0	0,050± 0	0,050±0	>3,125	0,006± 0	0,006±0	0.024±0
Malaleuca alternifolia	0,195± 0	0,390± 0	0,390±0	>3,125	0,162± 0,056	0,098±0	0.012±0
Malaleuca leucadendron	0,780± 0	0,780± 0	0,195±0	>3,125	0,162± 0,056	0,195±0	0.024±0
Eucalyptus globulus	0,390± 0	0,780± 0	0,195±0	>3,125	0,098± 0	0,780±0	0.024±0
Mentha piperita	0,292± 0,137	0,390± 0	0,195±0	>3,125	0,074± 0	0,050±0	0.012±0
Lavandula angustifolia	0,780± 0	>3,125	0,195±0	>3,125	0,292± 0	0,390±0	0.012±0
Lavandula hybrida	0,780± 0	1,560± 0	0,195±0	>3,125	0,098± 0	0,098±0	0.012±0
Cinnamomum zeylanicum	0,018± 0,008	0,012± 0	0.003±0	0,082±0, 027	0,030± 0	0,012±0	0.003±0
Rosmarinum officinalis	2,342± 1,106	1,560± 0	0,780±0	>3,125	0,104± 0,087	0,390±0	0.006±0
Citrus limon	2,342± 1,106	3,125± 0	0.390±0	>3,125	0,325± 0,405	0,390±0	0.003±0
Thymus vulgaris	0,05±0	0,098± 0	0,024±0	>3,125	0,012± 0	0,024±0	0.0015±0

P31

Systematic Review and Meta-Analysis on Knowledge Attitude and Practices on African Animal Trypanocide Resistance

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Introduction: African trypanocide resistance is an emerging public health emergency whose control requires a revisit on farmer's knowledge, attitudes, and practices in developing countries. African animal trypanocide resistance (AATr) is rife in an environment where drug use and policy decisions are disjointed. The objective of the study was to identify community factors responsible for the development of AATr. This was important since diminazene aceturate (DA), isometamidium chloride (ISM), and homidium bromide (HB) have existed for over 30 years and no new drugs have been provided to farmers.

Methods: An electronic keyword search across 12 databases was conducted using a search criterion from 1806 to June 2022. This generated a total of 24 publications, but after removing duplicates, review articles, and nonrelated articles, a total of eight papers were included in the analysis by following the PRISMA checklist. A meta-analysis was conducted on the data extracted and the risk ratio and inverse variance at 95% confidence interval were calculated using RevMan®.

Results: All the eight articles in the study showed that DA was the most preferred trypanocide in both West and Eastern Africa. Poor farmer knowledge of AATr and limited drug options were major drivers for trypanocide resistance. In addition, farmer treatments, use of untrained personnel, poor administration, poor dosing, and preparation of trypanocides were major drivers for the development of AATr and similarities were identified in DA and ISM practices ($P = 0.13$).

Conclusions: AATr is spread in developing countries due to a lack of community knowledge, attitudes, and drug-use practices. This situation could be reversed through interdisciplinary collaborations in endemic communities by promoting effective treatments and responsible drug handling.

P32
Formulations based on essential oils for the environmental microbial control.

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Introduction:

Resistant pathogenic microorganisms in the air are major threats to human health. Antimicrobial effectiveness of essential oils (OEs) and their vapours are important for the fight against drug resistance and environmental microbial control. The objective of the study was to evaluate the effectiveness of single or mixed EOs (M-EOs) to control the growth of bacterial and fungal environmental/hospital strains.

Methods:

15 OEs were tested against 3 bacterial resistant strains (*Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and 6 fungal strains (*Aspergillus fumigatus, Aureobasidium pullans melanigenum, Cladosporium cladosporioides, Alternaria alternata, Chaetomium globosum, Candida auris*). Broth micro-dilution tests according to EUCAST guidelines were used to obtain MIC and MBC values. The most active EOs were formulated in mixtures and the antimicrobial efficacy of their volatile compounds was evaluated using both micro-atmosphere tests and confined nebulization models. Starting from a suitable bacterial or fungal suspension, the charge variation was evaluated by making 2 nebulisations/minute for 10 or 20 minutes in an environment of 0.8 m^3. The air quality was assessed by sampling on sorbent tubes followed by thermal-desorption and GC-MS analysis.

Results:

After a single nebulization, data show a statistically significant reduction (between 50% and 80%) depending on the bacterial species, and a time-dependent inhibition of fungal growth (between 77% and 95%). Furthermore, the quality analysis does not identify concentrations of volatile compounds harmful to the human health.

Conclusions:

Results indicate that air treatment by M-EOs nebulization is a possible, green, and safe treatment against hospital strains and antibiotic resistance in line with the one health approach.

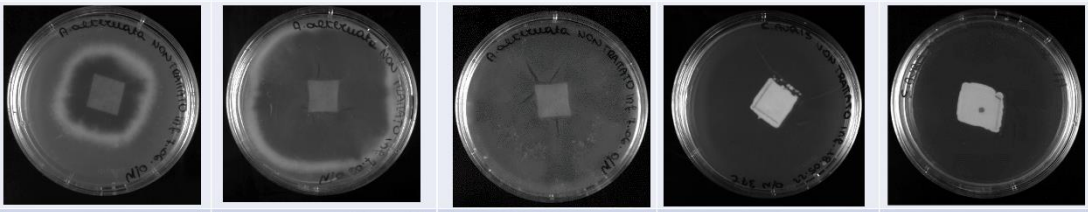
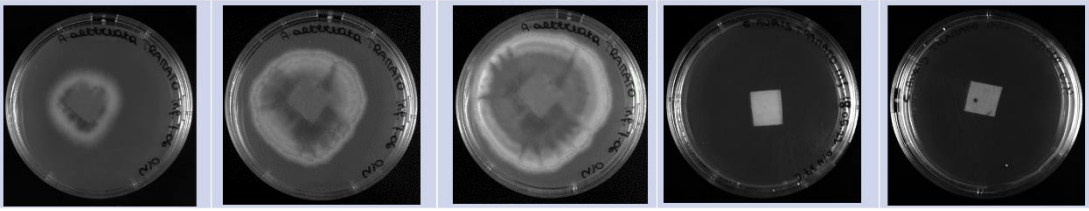
	Alternaria alternata			*Candida auris*	
	3d	5d	7d	24h	48h
NT					
TR					

Figure 1: Confined nebulization models against *Alternaria alternata*, and *Candida auris*. NT: Non treatment sample, TR: treated sample. Figures show microbial growth after 3,5,7 days, and 24,48 hours.

P33
Characterization of third generation cephalosporin resistant Enterobacteriaceae from healthy pigs in western region of Cuba

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Introduction:

Third-generation cephalosporin-resistant (3GCR) Enterobacteriaceae have been reported worldwide in food-producing animals and are regarded as a significant reservoir of antimicrobial resistance. However, little is known about the situation in Cuba. This study aimed to gain insight into the characteristics of 3GCR Enterobacteriaceae isolated from pigs in Cuba.

Methods:

Rectal swabs from 90 healthy pigs were taken from 9 different farms in the western region of Cuba and inoculated on MacConkey agar supplemented with 2 µg/ml of cefotaxime. Bacteria were identified by MALDI-TOF and antimicrobial susceptibility to 11 antibiotics was determinated by agar disk diffusion test according to the CLSI criteria. Twenty-two isolates were further characterized by whole genome sequencing using Oxford Technologies' nanopore and subsequent *in silico* analysis.

Results:

Thirty-six samples (40%) originating from 9 farms were positive for 3GCR Enterobacteriaceae, 34 isolates were identified as *Escherichia coli* (*E. coli*) and two as *Enterobacter hormaechei*. The most frequent extended-spectrum β-lactamase (ESBL) genes were *bla*$_{\text{CTX-M-32}}$ (n = 18) followed by *bla*$_{\text{CTX-M-15}}$ (n = 2), *bla*$_{\text{CMY-2}}$ (n = 1) and *bla*$_{\text{ACT-7}}$ (n = 1). Eleven different Inc types were found in 17 isolates and IncFIB (n = 8) was the most predominant. Only for 2 isolates the *bla*$_{\text{CTX-M-32}}$ and *bla*$_{\text{CTX-M-15}}$ genes were localized on IncN and IncFIB plasmids respectively.

Conclusions:

The results show the dominance of *bla*$_{\text{CTX-M-32}}$ gene. The *bla*$_{\text{CTX-M-32}}$ gene carrying *E.* coli isolates were genetically diverse, belonging to 13 different sequence types, suggesting multiple sources of acquisition of *bla*$_{\text{CTX-M}}$ genes. The prevalence of 3GCR Enterobacteriaceae needs further evaluation in other regions of Cuba.

P34
Recurrent urinary tract infection in pets and dynamics of antibiotic resistance in Enterobacteriaceae

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Introduction:
The increasing emergence of multidrug-resistant bacteria, causing urinary tract infections (UTI) in dogs and cats, represents a therapeutic threat as well as a public health concern.

Methods:
30 pets (24 dogs and 6 cats) urines were submitted repeatedly to bacteriology. All isolated strains were subjected to minimum inhibitory concentration method to assess antimicrobials susceptibility.

Results:
71 isolates from UTI were identified as follow: 45 E.coli (16 dogs and 2 cats, respectively), 4 Proteus spp. (1 dog and 1 cat respectively), 18 Klebsiella spp. (6 dogs and 1 cat respectively), 2 Enterobacter spp. (2 cats), 2 Serratia spp. (1 dog).8% of the isolates show a totally sensitive MIC. Extended spectrum beta-lactamase (ESBL) and/or AmpC-encoded cephalosporinases were detected in 24 strains: 9 ESBL/AmpC, 14 ESBL+, 1 AmpC.Most of these strains were also resistant to more than three classes of antibiotics (Amphenicols, Fluoroquinolones,Sulphonamides, Tetracyclines, Aminoglicosydes).
In two dogs, ESBL and/or AmpC properties were acquired during the UTIs recurrency and in other three cases AmpC properties were lost (one dog and two cats).

Conclusions:
Pets can serve as reservoir/source of ESBL and AmpC-encoded cephalosporinases producers. Thecorrect approach based on bacteriology and susceptibility testing followed by a prudent and
appropriate use of antibiotics seems to reduce the persistence of ESBL and AmpC genes. On the other hand, this report should be a warning that also pets have to be a role in multiresistant bacteria as well as food-producing animals. This work is funded by Italian Ministry of Health (14/2019 RC

P35
First detection of the oxazolidinone resistance *poxtA2* and *cfr*(D) genes in Streptococcus dysgalactiae subsp. equisimilis of swine origin, Italy

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Introduction:
Streptococcus dysgalactiae subsp. *equisimilis* (SDSE) is a pathogen responsible for infections in humans and livestock. Oxazolidinones are last resort antimicrobials only approved for clinical use and not administered in veterinary medicine. However, the use of phenicols in livestock might also select for oxazolidinones resistance genes. Recent data indicate the presence of *poxtA*, an oxazolidinone resistance gene, in enterococci isolated from Italian pig herds. In this study, we first identified a SDSE isolate of porcine origin that harbors *poxtA2* and *cfr*(D) genes.

Methods:
The SDSE strain was isolated from the brain of a pig affected by polyserositis in 2020. The genome was sequenced by a hybrid process using both short-reads Illumina and long-reads MinION.

Results:
Antimicrobial susceptibility testing revealed that SDSE was resistant to tetracycline, erythromycin and florfenicol, while susceptible to linezolid, tedizolid, chloramphenicol and vancomycin. Bioinformatic analysis showed the presence of a new plasmid, named pSdyV305, which resulted from a recombination event between the pSDSE159 plasmid of SDSE159 and a *cfr(*D)/*poxtA2*/*fexA*-carrying region of the pV386 plasmid of the porcine *Enterococcus faecalis* V386. Co-location of the *poxtA2* and *cfr*(D) genes was previously detected in *E. faecalis* and *E. casseliflavus* isolates from Italian pig herds. Interestingly, in the farm of origin, florfenicol had been used in the two years before the SDSE isolation, confirming the importance of the selective pressure generated by phenicols.

Conclusions:
The detection of *poxtA2* and *cfr*(D) in a potential zoonotic agent and in a new bacterial genus poses a serious threat to public health, worldwide.

P36
Occurrence, types and mobility of CTX-M genes in Gram-negative bacteria isolated from imported retail seafood products

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Introduction:
There is growing concern about the spread of extended-spectrum β-lactamases (ESBL) via contaminated seafood products. Here, we investigated occurrence, type and mobility of ESBL genes in seafood imported to the UK.

Methods:
288 samples of imported seafood were collected from three retailers in London, including shrimp and catfish from Vietnam and seabass from Turkey (n=96 samples each). After enrichment in MacConkey broth supplemented with 1 µl/ml cefotaxime, cultures were plated on ESBL selective plates (Brilliance-ESBL, Thermo Scientific). ESBL genes belonging to the CTX-M-1 and CTX-M-9 groups were detected by high-throughput real-time PCR (Fluidigm), and positive isolates were characterized by whole-genome sequenced (WGS) and conjugation experiments.

Results:
88 (31%) of the 288 samples yielded presumptive ESBL-producing bacteria, of which 29 (10%) were CTX-M-positive by PCR and WGS, Including 7 *Klebsiella pneumoniae*, 6 *Citrobacter freundii*, 6 *Enterobacter cloacae*, 5 *Escherichia hermannii*, 2 *Moraganella morganii*, 2 *Proteus vulgaris* and 1 *Escherichia coli*. The ESBL genes involved were CTX-M-55 (n=10), CTX-M-15 (n=7), CTX-M-27 (n=7) and CTX-M-14 (n=4) with a single isolate carrying both CTX-M-15 and CTX-M-27. Out of 22 strains tested by conjugation, 15 (68.2%) were able to mobilize CTX-M genes to laboratory *Escherichia coli* K12.

Conclusions:
Our findings confirm that imported seafood products pose a potential risk for consumers due to the frequent occurrence of ESBL genes of high clinical importance such as CTX-M-15. This risk of transmission is concrete in view of the growing tendency to consume raw seafood.

P37
Following up the epidemiology of 3rd generation cephalosporin resistant Enterobacterales in individually marked piglets

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Introduction:

Faecal dynamics of 3rd generation cephalosporin resistant Enterobacterales (3GCRE) in 41 piglets of three sows raised antibiotic-free was followed through six sampling (1, 23, 35, 62, 92,137 days of age).

Methods:

Isolates were recovered on eosin-methylene-blue agar with 2 mg/l cefotaxime, identified using MALDI-TOF and their antibiotic susceptibility tested. Twenty-four isolates from piglets with multiple positive samples were selected for whole genome sequencing on an Oxforde Nanopore MinIOn platform.

Results:

The sows appeared negative in the first sampling, but in the second sampling 2/3 became positive. Prevalence in piglets decreased with age; first, second (with sow), third and fourth (in piggery) sampling yielded 50, 20, 25 and 2 isolates in 30, 19, 21 and 2 piglets, respectively, none in the fifth and sixth sampling. Twenty-one piglets were positive more than once. Ninety *Escherichia coli* and ten *Citrobacter freundii* were identified (including sow isolates), 71 were ESBL and 29 were AmpC producers. ST58 carrying $bla_{CTX\text{-}M\text{-}55}$ was the most prevalent (n=5, cgMLST45951), followed by ST2952 carrying $bla_{CTX\text{-}M\text{-}32}$ (n=4, cgMLST149190). We found $bla_{CTX\text{-}M\text{-}1}$ producers (n=5) of various STs, two carried $bla_{CMY\text{-}67}$ and one $bla_{CMY\text{-}113}$ simultaneously with $bla_{CTX\text{-}M\text{-}1}$. Two piglets carried the same *E. coli* ST453 across multiple samplings, while similar *E. coli* strains were found in different piglets in the same sampling.

Conclusions:

Sows were negative at the start and piglets carried different 3GCRE in different samplings, which indicate that piglets frequently obtain 3GCRE from the environment, however, transfer amongst piglets is probable. 3GCRE carriage proved to be transient in absence of antibiotic use.

P38

The veterinary hospital as a cross infection hub of multi drug resistant enterobacterales

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Background: Extended-spectrum beta-lactamase producing Enterobacterales (ESBL-PE) are common in various reservoirs and pose a treatment challenge. Previous studies demonstrated high carriage rates among pets and a potential role of spread to humans. ESBL-PE are likely to be cross-transmitted and ubiquitous in the hospital environment.

Objectives: We aimed to determine the prevalence of ESBL-PE among staff, environment and animals treated in University Veterinary Hospital in Israel between 2021 and 2022.

Methods: Staff members were sampled using rectal swabs. Pets brought to the hospital were sampled following owners' consent. Foals were always hospitalized with their healthy mothers. Environmental samples were collected from sinks, keyboards and medical devices. ESBL-PE were recovered following enrichment in liquid broth followed by culture on ESBL-selective agar and identified by MALDI-TOF.

Results: 164 positive samples yielded 238 isolates, most common was *Escherichia coli* (n=105) and *Klebsiella* species (n=54). Carriage rates were 33% among staff, 74% in animals, and 89% in the environment. Highest prevalence was in horses (84%). High mother-foal concordance was noted.18 of 20 couples shared at least one isolate. Several strains were apparently clustered.

Conclusions: ESBL-PE carriage rates in a veterinary hospital were high among animals and environment. Prevalence among staff is higher than historic population cohorts. Further studies are required to determine genetic relatedness, causality and dynamics following infection control measures. These very high rates demonstrate the importance of holistic approach regarding human-animal interface and the role of the medical facility as a cross-infection hub.

P40

Effect of antimicrobial therapies on gut microbiome resistance profiles during an outbreak of post-weaning diarrhoea

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Introduction:
Porcine post-weaning diarrhoea (PWD) is economically relevant for the swine industry, as it severely affects intestinal health and growth of piglets. Antimicrobials are commonly prescribed for its treatment. The objective of this study was to determine the effect of common treatments of PWD on antibiotic resistance (AMR) profiles of pig gut microbiome.

Methods:
A total of 210 piglets were transferred from a farm with recurrent problems of PWD to an experimental farm and divided into six different treatment groups: trimethoprim/sulphonamide, colistin, oral commercial vaccine, gentamicin, control with water acidification, and untreated control. An extra group remained at the farm of origin following the implemented amoxicillin treatment. Faecal samples were collected at four different sampling times: nursery (ST1), three days (ST2), two weeks (ST3), and four weeks (ST4) post-treatment. A total of 280 samples (10 animals/group/ST) were selected and shotgun metagenomics was performed. From assembled contigs, AMR gene profiles were identified with ABRicate using the curated Comprehensive Antibiotic Resistance Database.

Results:
Preliminary results identified significant differences in richness of AMR genes between STs for all treatments. No differences were observed among groups at ST1, ST2, and ST4. Significant differences were identified at ST3, displaying larger richness in the gentamicin and the untreated control in comparison to the rest of the groups, notably to the control with water acidification. Most abundant identified AMR genes are known to confer resistance to macrolides, lincosamides, tetracyclines, and fluoroquinolones.

Conclusions:
Overall, this study provides insight for a more appropriate therapy selection to reduce AMR prevalence during a PWD outbreak.

P41
Antimicrobial use restrictions in dairies: What are the implications for producers and veterinarians?

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Introduction:

Restricting the use of antimicrobials in animals does not only impact the animals, but also producers and veterinarians. Therefore, the objectives of the study were to identify barriers, facilitators and consequences for dairy farmers and veterinarians of the implementation of a new regulation restricting the use of antimicrobials of very high importance in human medicine (3rd and 4th generation cephalosporins, fluoroquinolones, and polymyxins; defined as category I antimicrobials by Health Canada) in animal production in the province of Quebec, Canada.

Methods:

Individual semi-structured interviews were performed with 15 veterinarians and 27 dairy producers. A thematic analysis based on the COM-B model was perform using NVivo 12.0.

Results:

Major barriers included the fear of economic consequences and the time it takes to get diagnostic test results. Long delays before treating an animal was considered by many producers as negatively impacting animal well-being. A few facilitators were also mentioned by producers and veterinarians such as access to previous training about antimicrobial use and resistance. Producers mentioned consequences related to the new regulation; some producers mentioned losing their income while others mentioned making more profit.

Conclusions:

Our results allow to better understand the economic, social and operational barriers and facilitators associated with the application of new regulations on antimicrobial use in dairy farming, which are important to consider before the development and implementation of future regulations.

P42
‘Brave Enough’: a qualitative study of veterinary decisions to withhold antimicrobials

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Introduction:

Veterinarians sometimes prescribe antimicrobials when they know they are definitely or probably not required. Reasons for not withholding antimicrobials are complex and likely to be highly context-specific. Understanding this phenomenon is a critical step in designing effective stewardship interventions.

Methods:

Semi-structured interviews were conducted with 22 veterinarians who treat companion animals in Australia. Themes were organised according to the Theory of Planned Behaviour.

Results:

Beliefs about the consequences of withholding antimicrobials (behavioural beliefs) were dominated by fears of clinical deterioration and of failing to meet client expectations, which could result in very serious consequences for the veterinarian and their practice. These fears were powerful barriers to withholding antimicrobials.

Normative beliefs (perceived approval or disapproval) related to their client, their employer and colleagues, the veterinary registration board and veterinary academics. Most participants mentioned clients presenting with an expectation of receiving antimicrobials and the challenge of withholding antimicrobials in these situations.

Beliefs about the difficulty of withholding antimicrobials (control beliefs) centred around client factors, most importantly their capacity to adequately monitor their animal, to pay for further investigations, or to undertake non-antimicrobial management at home. Workplace factors, including time pressure and availability of other clinic staff to help with investigations, also played a role.

Important background factors included the veterinarian’s communication skills and self-confidence, habits and energy levels. Client health literacy and the veterinarian-client relationship were also important.

Conclusions:

Withholding antimicrobials can be extremely difficult for veterinarians, but there are modifiable factors that could be leveraged to curb unnecessary antimicrobial use.

P43
Familiarity, fear, finances and fractious felines: exploring non-clinical drivers of veterinary antimicrobial choices

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Introduction:

Previous analyses have shown that the most common antimicrobial choices for dogs and cats in Australia are amoxycillin-clavulanate and cefovecin, whereas low-importance antimicrobials, such as amoxycillin and trimethoprim-sulfonamides, are rarely used. The reasons for this are unclear.

Methods:

Semi-structured interviews were conducted with 22 veterinarians who treat companion animals.

Results:

Few participants were familiar with the Australian Veterinary Prescribing Guidelines or antimicrobial importance ratings.

Broader-spectrum antimicrobials, particularly amoxycillin-clavulanate, were seen as 'safer' empirical choices than narrower-spectrum antimicrobials. Veterinarians' longstanding familiarity with broad-spectrum antimicrobials, and the effectiveness of these choices in the Australian context, were major barriers to the use of narrower spectrum antimicrobials, even when recommended in guidelines. Veterinarians feared narrower spectrum antimicrobials would be less effective, resulting in unwell animals and dissatisfied clients.

Many veterinarians reported that their practices did not hold stocks of narrower-spectrum antimicrobials, further reinforcing their habit of using broader-spectrum agents.

Minimising costs for the client was often important, especially for clients with financial constraints and/or larger dogs. This consideration, and practical difficulties of achieving the correct dose for the patient's body weight (e.g. half a capsule) sometimes resulted in suboptimal antimicrobial choices.

Most participants were aware that fluoroquinolones and later generation cephalosporins, e.g. cefovecin, should be used sparingly. Nevertheless, fractious cats and owners with poor capacity to give oral medication often left veterinarians feeling there was no alternative to administering a long-acting cefovecin injection.

Conclusions:

Antimicrobial stewardship initiatives must address the non-clinical factors that influence veterinary prescribing choices, including habit, fears and practical challenges.

P44
Balancing effectiveness and AMR risk: a novel method to select rational empirical antimicrobial therapy

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Introduction:

The formula for rational selection of empirical antimicrobial therapy (FRAT) published by Blondeau and Tillotson combines prevalence and antimicrobial susceptibility results to estimate an antimicrobial's effectiveness in a given clinical syndrome. This method inherently favours broader-spectrum antimicrobials. A more nuanced method is required to evaluate empirical therapy options.

Methods:

MCAS results from canine and feline urinary isolates were obtained from laboratories in Australia and Portugal.

To both data sets, we applied the FRAT and our own novel method, a whole population antimicrobial simulation, which incorporates antimicrobial importance ratings as a proxy for antimicrobial resistance risk and results in an 'antimicrobial cost per cure'.

Results:

Results from 6196 urinary isolates from Australian cats and dogs and 4990 from Portugal were analysed. Susceptibility was significantly lower to all antimicrobials for Portuguese isolates than for Australian isolates ($p<0.001$).

FRAT suggested amoxycillin-clavulanate to have the highest impact factor for Australian dog (95) and cat (95) UTIs, whereas amikacin had the highest impact factor in Portuguese dogs (85) and cats (83). Both drugs are WHO critically important antimicrobials. Our simulation rated trimethoprim-sulfa (TMS) as the optimal first-line choice in both countries, in accordance with international guidelines. The TMS ‘costs per cure’ were lower in Australia (1.15 in dogs, 1.21 in cats) than in Portugal (1.47, 1.47).

Conclusions:

Our novel whole-population antimicrobial simulation balances expected clinical effectiveness with antimicrobial resistance risk and provides a useful alternative to FRAT.

Acknowledgments: We thank ASAP Laboratory (Australia), INNO Veterinary Laboratory (Portugal) and Dr Andreia Garces for their assistance.

P45
Understanding the use of sex pilus specific bacteriophages to reduce conjugative dissemination of antibiotic resistance

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Introduction:
The aim of this study is to isolate novel sex pilus targeting (SPS) phages to tackle AMR bacteria.

Methods:
Fifteen SPS phages were isolated, eleven were ssDNA and four were ssRNA phages. All phages were able to infect strains with the F plasmid but there was some phage plasmid specificity observed on different F like plasmid field isolates.

Results:
Phylogenetic analysis of the 11 ssDNA phages genomes and protein pIII confirmed that they belong to genus *Inovirus* and are highly similar to filamentous (Ff) phages. Two of these phages only demonstrated a similarity of <95% to any of the Ff reference strains at the nucleotide level which may represent a new species of *Inovirus*. Three of the ssRNA phages were highly similar to genus *Emesvirus* and the remaining ssRNA phage was similar to *Qubevirus*. Growth analysis of selected ssDNA and ssRNA phages on strains containing derepressed and repressed plasmids demonstrated that growth on derepressed plasmid hosts had a short latent period and large burst size compared to a longer latent period with shorter rise period and burst size observed on repressed plasmid hosts. Selected phages demonstrated ca. 60% plasmid loss on derepressed hosts. Selection was limited on more repressed plasmid hosts, with a minimum loss of 0% and a maximum loss of 14%.

Conclusions:
These results suggest that treatment with phage will have variable efficacy in reducing plasmid borne AMR and that repression may pose a limit on the effectiveness of this approach to treat or reduce infections by targeting the plasmids.

P46
Upper and lower respiratory tract microbial biomarkers associated with healthy pigs

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Introduction:

A decade of microbiome studies has linked respiratory disease in pigs, collectively named Porcine Respiratory Disease Complex (PRDC), to an alteration in the respiratory microbial community, especially of the upper respiratory tract. Here, we aimed at identifying bacterial and fungal biomarkers in the upper and lower respiratory tract of pigs associated with the animal health status.

Methods:

35 pigs were sampled by nasal and tracheobronchial swabbing during a PRDC outbreak at a conventional Danish pig farm. Bacterial microbiota was analysed by 16S rRNA gene sequencing, and the fungal microbiota of nasal samples was profiled using ITS rRNA gene sequencing. Sampling, extraction and run controls were also included. Data analysis was performed in R.

Results:

Upon clinical examination, 25 animals presented clinical signs of PRDC (coughing score), whereas 10 were healthy. Although no differences were observed in microbial diversity and richness between healthy and diseased pigs, differential abundance and linear discriminant analyses identified different taxa that were significantly associated with healthy individuals (p-value <0.05). One ASV assigned to *Pediococcus pentosaceus*, a member of lactic acid bacteria with known probiotic effects, was invariably correlated to the nose and trachea of healthy pigs. In the nose, three fungal species were associated with healthy animal, including the probiotic candidate *Wickerhamomyces anomalus* (previously *Pichia anomalus*). In the trachea, *Aerococcus viridans* and *Kurthia spp.* were correlated with healthy animals.

Conclusions:

We identified microbial taxa significantly associated with healthy respiratory tract, which can be further investigated as probiotic candidates for PRDC prevention.

P47
Hemp extract seed oil antimicrobial activity against *Staphylococcus pseudintermedius* and *Pseudomonas aeruginosa* strains isolated from pyoderma and external otitis in dogs

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Introduction:
Scientific studies have shown that Hemp (*Cannabis sativa*) and its extracts as essential and seed oil possess antimicrobial properties against both Gram-positive and Gram-negative bacteria. These findings make Hemp and its derivatives promising candidates for the future development of innovative antimicrobial therapies against bacteria of veterinary clinical interest as multi-resistant *Staphylococcus pseudintermedius* strains and *Pseudomonas aeruginosa* strains, responsible for serious infections in dogs. The aim of this study was to evaluate *in vitro* the antimicrobial activity of Hemp extract seed oil against *Staphylococcus pseudintermedius* strains and *Pseudomonas aeruginosa* strains isolated from canine pyoderma and external otitis.

Methods:
The antimicrobial activity of Hemp seed oil was evaluated by broth-microdilution to determine the minimum inhibitory concentration (MIC) in ten methicillin-resistant *S. pseudintermedius* strains and ten *P. aeruginosa* strains. The Hemp seed oil used in this study was characterized by the absence and tetrahydrocannabinol (THC) and very low value of cannabidiol (CBD).

Results:
The Hemp seed oil showed higher activity against *S. pseudintermedius* strains than *P. aeruginosa* strains. The MIC value for all *S. pseudintermedius* and *P. aeruginosa* was indeed 0.05% and >0.2%, respectively.

Conclusions:
CBD and THC are widely reported to be responsible for the antimicrobial capacity of Hemp extracts. Our results highlight that Hemp seed oil even without THC and low CBD has antimicrobial properties against *S. pseudintermedius* strains. Further studies will be needed to unveil the mechanisms underlying antibacterial activity of CBD and THC free-Hemp seed oil and to establish its potential as topical treatment for skin infections.

P48
Global AMR funding landscape with a One Health lens

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Introduction:

The Global AMR Research and Development (R&D) Hub (HUB) was launched in May 2018 following a call from G20 leaders to address challenges and improve co-ordination and collaboration in AMR R&D. Here we outline how the HUB is supporting global priority-setting and evidence-based decision-making on allocation of resources for AMR R&D across all One Health sectors.

Methods:

The HUB's Dynamic Dashboard collects and presents information on public and philanthropic investments in AMR R&D globally since 2017. The data is regularly updated and analyzed to identify potential gaps and opportunities in AMR R&D.

Results:

As at 30th January 2023, the Dynamic Dashboard presents information on 12,931 projects worth 10.2 billion USD. Work to date has highlighted gaps and opportunities for veterinary vaccines to reduce antibiotic use and the state of R&D investments in animal health in low and middle income countries. A limited amount of funding targets animal, environment and plant health sectors (10%, 4% and 1%) compared to human health (85%), with cross sectoral investments comprising 6% of the total funding captured by the Dynamic Dashboard.

Conclusions:

As a global knowledge centre and driving force for AMR R&D across the One Health continuum, the HUB continues to provide a global snapshot of the AMR R&D activities, offering an evidence base to foster global priority setting at the highest political levels and decision making on the allocation of resources for AMR R&D.

P49
Behavioural drivers of antimicrobial consumption among farmers from rural Western Uganda: a questionnaire-based survey

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Introduction:

Antimicrobial resistance (AMR) is a leading global human and animal health threat. However, surveillance and control measures for drug resistance in low- and middle-income countries (LMICs) are limited.

Methods:

We conducted a questionnaire-based survey from November 2021 to February 2022 to evaluate antibiotic use by two-hundred farmers in three regions from rural western Uganda. The questionnaire included four categories: farmer's personal information, herd's information, antibiotic use in animals and behavioural patterns of antibiotic consumption by farmers.

Results:

According to farmers' answers, penicillin, tetracyclines and sulphonamides were the most frequently used antimicrobials. 17% of the farmers admitted starting the treatment of their livestock before consulting a veterinarian. Furthermore, 45.9% recognized not to wait the withdrawal period before slaughter or consuming milk from treated animals. Regarding farmers' antibiotic use, 66.7% admitted they commonly acquired antibiotics in drug stores without medical prescription, and 13.5% recognized to self-medicate with antibiotics prescribed to their livestock by the veterinarian.

Conclusions:

A high proportion of the farmers surveyed showed an irresponsible use of antibiotics in both, animals and humans. Factors such as misinformation, lack of education and inaccessibility to health care and diagnostic facilities make people from LMICs more exposed to self-medication, lack of access to effective antibiotics, intake of sub-inhibitory doses of antimicrobials, drug sharing with friends and neighbours, or use of low quality or expired drugs. There is an urgent need for guidelines and awareness about AMR to reduce self-medication an inappropriate use of antibiotics in rural settings from Uganda.

P50
First detection of SCC*mec-mecC* hybrid element in methicillin-resistant *Mammaliicoccus lentus* (MRML) from camels in Algeria

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Introduction:

Mammaliicocci are considered to be opportunistic pathogens. They have been shown to carry several antimicrobial resistance genes, especially *mecA*, leading to resistance to nearly all beta-lactam antibiotics. Here, we characterized here *M. lentus* isolates from camels in Algeria harboring both *mecA* and *mecC*.

Methods:

Methicillin resistant *M. lentus* "MRML" isolates were obtained from camel nasal swabs using selective media. Bacterial identification was performed using MALDI-TOF MS. The presence of *mecA* and *mecC* was confirmed with PCR. Also, whole-genome sequences were obtained using the MinION long-read sequencing platform. DNA analysis was performed using different bioinformatics tools.

Results:

Six *M. lentus* isolates were recovered from camels. Five *M. lentus* isolates were *mecA*- and *mecC*-positive, while one isolate carried only *mecA.* Former five *M. lentus* were very similar and seemed to be clonally related, showing minimum 9 and maximum 47 SNP differences. *mecA/mecC*-positive isolates carried a SCC*mec-mecC* hybrid element highly similar (99.98%) to that of the *M. sciuri* GVGS2 strain (Figure 1), with a *mec* complex type A (*mecA, mecR1, mecI*) that is closely related to the SCC*mec* type VII, and the *mecC* region(*mecI-mecR1-mecC-blaZ*), as present in *S. aureus. M. lentus* isolates carried multiple other resistance genes responsible for penicillin (*blaZ*), lincosamides and macrolide (*erm(B), mph(C)*), aminoglycoside (*str*), and tetracycline (*tet*(K)) resistance.

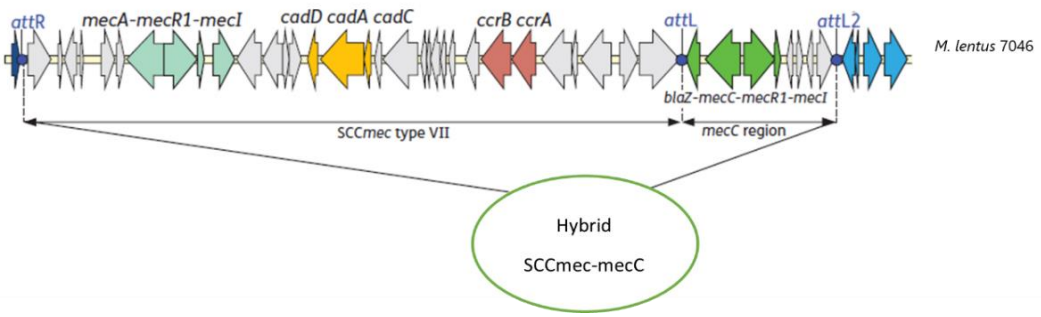

Figure 1: Schematic representation of the hybrid SCC*mec-mecC* element in *M. lentus.*

Conclusions:

This study demonstrates the further dissemination of the previously described hybrid SCC*mecA/C* in mammaliicocci in food producing animals in Northern Africa, representing a new potential reservoir for methicillin resistance in livestock.

P51
Modelling the holistic societal cost of antimicrobial resistance and interventions to tackle it

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Introduction:

The need to approach AMR from a cross-sectoral perspective is increasingly widely acknowledged. However, research into the economics of AMR tends to focus on the direct human health effects, and methods for capturing broader One-Health-economic impacts are not widely adopted.

Methods:

In response, we created the Agricultural Human Health Micro-Economic (AHHME) modelling tool. Using state transition models, it calculates the cost-effectiveness of antimicrobial stewardship interventions in agriculture. It takes into account the effect on: agricultural productivity, life years lost, labour productivity, and healthcare costs, and can be adapted to a range of interventions outside agriculture.

Results:

In our recent paper, we use AHHME to simulate a hypothetical reduction in antibiotic use in livestock. Over 10,000 simulations, we estimated that countries should be willing to pay a median of $0.83 to $7.94 USD per person per year to achieve this, depending on country income level. Only in the most pessimistic scenario was the intervention not worth implementing at any cost.

Conclusions:

Our model allows for more accurate design, prioritisation, and evaluation of national AMR policies. Our results reaffirm the particular importance of stewardship in middle-income countries, and suggest that the effects of AMR are considerably underestimated when focusing on direct human health impacts.

This approach can be adapted to estimate the total burden of AMR in a country, which is currently being piloted as ‘AHHME-Burden’ with colleagues in Zambia and Malawi. We support open-source science: our code, a guide on adapting it to one’s own country context, and an interactive app, are available on our GitHub.

P52
Setting epidemiological cut off values (ECOFFs) for veterinary pathogens – a step towards clinical breakpoints

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+ on behalf of the COST Action ENOVAT (European Network for Optimization of Veterinary Antimicrobial Treatment)

Introduction:

Antimicrobial resistance is one of the greatest challenges of the 21st century. Antimicrobial susceptibility testing (AST) plays a key role as a rational basis for targeted antimicrobial therapy. Unfortunately, AST in veterinary medicine is hampered by the lack of clinical breakpoints (CBPs) for relevant antimicrobials and bacterial species. An important prerequisite for establishing a CBP is the availability of an epidemiological cut-off (ECOFF). Therefore, members of VetCAST and the COST Action ENOVAT have started a joint project to fill some of these gaps.

Methods:

We describe the requirements of the European committee on antimicrobial susceptibility testing (EUCAST) for setting new ECOFFs (EUCAST, SOP 10.2), which is based on aggregated minimum inhibitory concentration (MIC) distributions using the broth microdilution method following ISO 20776-1 and inclusion of EUCAST recommended QC reference strains. The test ranges should be sufficient to cover the complete MIC range of the wild-type population. At least five laboratories must contribute to the aggregated MIC distributions to get at least 100 wild-type minimum inhibitory concentration (MIC) distributions.

Results:

Comprehensive data on the MIC distributions of six veterinary pathogens (*Staphylococcus aureus, Staphylococcus pseudintermedius, Streptococcus equi, Actinobacillus pleuropneumoniae, Pasteurella multocida, Mannheimia haemolytica*) and eight antimicrobials, including penicillin, aminopenicillins and tetracyclines as well as of reference strains for quality control were generated. Selected MIC distributions are used as examples to show which data sets are suitable for ECOFF determination and which do not meet the requirements.

Conclusions:

The ECOFFs derived will be added to the EUCAST database and used to establish veterinary CBPs highly needed by diagnostic laboratories.

P53
Title: Implementation of WGS analysis within AMR monitoring reveals clonal spread of ESBL-producing E. coli in livestock and meat

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Introduction

Changes in EU legislation implemented in 2021 allowed the use of whole-genome sequencing (WGS) for monitoring of ESBL-producing *Escherichia coli* from livestock and meat, replacing phenotypic testing. We aimed to confirm the resistance phenotypes for a panel of antimicrobials, while also analysing the molecular epidemiology of ESBL-producing *E. coli*.

Methods

Selective culturing and typing of *E. coli* suspected of ESBL/AmpC-production (ESBL/AmpC-*E. coli*) from caecal content and meat was performed according to protocols of the European Reference Laboratory for Antimicrobial Resistance. Antimicrobial susceptibility was determined with broth micro-dilution using European standardised antimicrobial panels.

Short-read WGS was performed on Illumina Nextseq followed by genotypic prediction using Resfinder and Pointfinder. WGS data were also used to determine the phylogeny of the isolates.

Results

ESBL/AmpC-*E. coli* were isolated from broilers (n=34), slaughter pigs (n=47), veal calves (n=103), and dairy cattle (n=42) as well as from chicken meat (n=47), beef (n=8), veal (n=14), pork (n=6), other meat (n=4), or from imported beef (n=1) and imported chicken meat (n=32).

The correlation between the measured and predicted antimicrobial-resistance phenotype was 90% or higher. A total of 15 clusters were detected, in which the total core genome contained 40 SNPs or less. No clusters were detected amongst samples across the different livestock sectors.

Conclusion

Further implementation of WGS analysis of ESBL/AmpC-producing *E. coli* within the AMR monitoring program of EU member states and global surveillance programs will contribute to determining the attribution of livestock in the prevalence of ESBL/AmpC-encoding *E. coli* in humans.