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Enteric bacteria from a One Health perspective: fecal indicators and antibiotic resistance

EBRAHEEM DAABAG ELMARGHANI

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Abstract
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The rise of antibiotic-resistant enteric bacteria poses a significant threat to global health, highlighting the need for a One Health approach. Enteric bacteria such as *Enterococcus faecalis* (*E. faecalis*), *Enterococcus faecium* (*E. faecium*), and *Escherichia coli* (*E. coli*) are key indicators of water quality and environmental risk to public health. Their pathogenicity and ability to acquire antibiotic-resistant genes (ARGs) through horizontal gene transfer (HGT) exacerbate the issue. Extended-spectrum β-lactamase-producing *E. coli* (ESBL-Ec) and vancomycin-resistant enterococci (VRE) are serious human and animal pathogens present in the environment, potentially transmitted between animals and humans. Paper I addresses the distribution of fecal indicator bacteria, specifically *E. faecalis*, *E. faecium*, and *E. coli,* including their multidrug resistance (MDR) levels in the Örebro River, Sweden, from 2010 to 2011. The results revealed that VRE and ESBL-Ec strains were detected, highlighting the spread of significant antibiotic-resistant bacteria in relatively unpolluted regions. Paper II investigated third-generation cephalosporin-resistant *E. coli* (3GCR-Ec) strains in 2023 in Fyrisån river, Uppsala, Sweden. Results showed significantly higher concentrations of 3GCR-Ec, including ESBL-Ec, at the Wastewater treatment plant (WWTP) compared to Lake Mälaren. Multi-locus-sequence types (MLSTs) associated with virulence and MDR ST69, ST10, ST1193, and ST1722 were reported at multiple sampling points, while ST131 was found only downstream of the WWTP in March and November. ARGs were detected in all isolates. Notably, 65% of isolates carried an ESBL gene, and 43% harbored $bla_{CTX\text{-}M\text{-}15}$, which is commonly associated with clinically relevant strains, indicating an urgent need to mitigate the spread of these pathogenic bacteria. Paper III, an in-silico analysis of Extended-spectrum cephalosporin-resistant *E. coli* (ESC-Ec) (collected in May 2011), was performed to examine the interconnectedness between humans and livestock in rural Cambodia. Evidence of both clonal transmission and HGT was observed. Twenty-three percent of isolates were similar strains clustering into ten core genome MLST, circulating among the sources and across households and villages. IncF plasmids were identified in 75.9% of isolates, likely facilitating ARG transmission across hosts. In conclusion, the findings of this thesis underscore the critical need for enhanced surveillance and interventions to mitigate the spread of antimicrobial resistance, particularly in low-income settings.

Keywords: One Health, Multidrug-resistant, Horizontal gene transfer, Phylogenetic, vancomycin-resistant Enterococci, Extended-spectrum cephalosporin-resistant E. coli, Extended-spectrum ß-lactamase-producing E. coli, CTX-M.

Ebraheem Daabag Elmarghani, Department of Medical Sciences, Infection medicine, Akademiska sjukhuset, Ingång 30, våning 1, Uppsala University, SE-751 85 Uppsala, Sweden.

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“It's the possibility of having a dream come true that makes life interesting."

- Paulo Coelho

This thesis is dedicated to my parents, wife, children, and mentors for their unwavering support and encouragement. Your belief in me has been my greatest strength

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Ebraheem Elmarghani,** Sirirat Poonlapthawee, Per-Erik Olsson and Jana Jass. Multi-Drug Resistant Fecal Bacteria in a Swedish Lake System. (*Manuscript*)

II. **Ebraheem D. Elmarghani**, Robert Spörndly, Evangelos Mourkas, Josef D. Järhult. Temporal and spatial variation of third-generation cephalosporin-resistant and ESBL-producing Escherichia coli in a Swedish urban water system. *Next Research, 100436 (2025)*

III. **Ebraheem D. Elmarghani,** John H.-O. Pettersson, Clara Atterby, Rachel A. Hickman, Sokerya Seng, Sorn San, Kristina Osbjer, Ulf Magnusson, Evangelos Mourkas, Josef D. Järhult. Genomic insights into ESBL- and pAmpC-producing Escherichia coli transmission between humans and livestock in rural Cambodia. *Journal of Medical Microbiology*, 2025. **74**(3): p. 001988.

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Abbreviations

3GCR-Ec	Third-generation cephalosporin-resistant *E. coli*
AmpC-Ec	AmpC-producing *E. coli*
AMR	Antimicrobial resistance
ARGs	Antibiotic-resistance genes
BHI	Brain Heart Infusion
CFU	Colony-Forming Unit
ddL	D-alanyl-D-alanine ligase
dWWTP	Downstream of the Wastewater treatment plant.
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
EPA	European Environmental Agency
ESBL-Ec	Extended-spectrum β-lactamase-producing *E. coli*
ESC-Ec	Extended-spectrum cephalosporin-resistant *E. coli*
E-test	Epsilometer test
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FIB	Fecal indicator bacteria.
gDNA	Genomic DNA
LB	Lysogeny Broth
MDR	Multidrug resistance
NSAIDs	Non-steroidal anti-inflammatory drugs
OD	Optical density
PCR	Polymerase chain reaction
upWWTP	Upstream of the wastewater treatment plant
USEPA	United States Environmental Protection Agency
vanA	Vancomycin resistance gene A
WGS	Whole-genome sequencing
WWTP	Wastewater treatment plant

Introduction

One Health concept

One Health is a collaborative, multisectoral, and transdisciplinary approach that aims to achieve optimal health outcomes by recognizing the interconnectedness between humans, livestock, agriculture, and their shared environment[1]. This framework addresses critical challenges such as zoonotic diseases (e.g., COVID-19, Ebola, and rabies)[2], antimicrobial resistance (AMR) driven by antibiotic misuse in humans and animals[3], and environmental changes, such as those driven by human-induced climate change, which can alter animal habitats and increase the likelihood of disease transmission between animals and humans[4]. Food safety and security are other aspects of disease transmission between humans, livestock, and crops[5].

The One Health concept addresses not only zoonotic diseases and AMR but also emphasizes the importance of collaboration across traditionally separate sectors such as human, animal, and environmental health[2]. Recent initiatives, such as the Quadripartite collaborations involving FAO, WHO, WOAH (World Organization for Animal Health), and UNEP (United Nations Environment Programme), are helping to bridge these gaps by promoting joint governance and coordinated action[6,7].

In recent years, One Health has gained more political and financial attention. Increased funding supports research that combines multiple disciplines, while ongoing efforts aim to incorporate One Health into global policies. For example, the Quadripartite One Health Joint Action Plan focuses on pandemic prevention, sustainable food systems, and climate action[6].

Despite progress, challenges remain. Authorities often struggle to coordinate across traditionally separate sectors, resulting in delays and inefficiencies in collaboration. Furthermore, civil society organizations are not always fully integrated into One Health initiatives, which limits the inclusivity and effectiveness of these efforts[8]. Experts suggest that interdisciplinary training programs can enhance cooperation and promote respect among different fields. Such efforts can strengthen the use of proven methods and ensure better coordination across human and animal health[9].

Enteric bacteria as a source of environmental contamination

Enteric bacteria, such as Enterococci and coliform bacteria, including *Escherichia coli* (*E. coli*), are normal inhabitants of the gastrointestinal tracts (GITs) of vertebrates, including humans[10]. Among the *Enterococcus* species known as human commensal bacteria, *Enterococcus faecalis (E. faecalis)* and *Enterococcus faecium (E. faecium)* are opportunistic pathogens found in immunocompromised patients[11,12]. In addition to *E. coli*, these two species are reported as causative agents of various diseases, including endocarditis, urinary tract infections, and gastroenteritis[13-15]. Furthermore, due to their high abundance in human and animal feces and their limited growth in aquatic environments, they are considered reliable indicators of fecal contamination for assessing water quality[16]. According to the US Environmental Protection Agency (USEPA), the maximum acceptable limits of enterococcal numbers in surface waters are 104 colony-forming units (CFU)/100 ml in marine waters and 61 CFU/100 ml in freshwater[17-19]. The European Environmental Agency (EEA) recommendation for acceptable levels of fecal indicators in inland waters is 400 CFU/100 ml for enterococci and 1000 CFU/100 ml for *E. coli* for the water to be considered "good quality"[20].

In addition to non-resistant *E. coli*, antibiotic-resistant strains are of increasing concern in environmental monitoring[21]. Third-generation cephalosporin-resistant *E. coli* (3GCR-Ec) is one of the significant groups of antibiotic-resistant bacteria reported to be widespread in the environment[22,23]. Among 3GCR-Ec, there are two important subgroups that each produce a specific type of β-lactamase: Extended-spectrum β-lactamase-producing *E. coli* (ESBL-Ec) and AmpC-producing *E. coli* (AmpC-Ec), which are collectively referred to as extended-spectrum cephalosporin-resistant *E. coli* (ESC-Ec)[24].

Distribution of enteric bacteria in LMICs and HICs

The distribution of enteric bacteria, such as *E. coli* and Enterococci, varies significantly between low- and middle-income countries (LMICs) compared to high-income countries (HICs). In LMICs, higher contamination levels are often observed due to inadequate sanitation infrastructure, limited access to clean water, and lower levels of public health education[25]. These factors contribute to the persistence and spread of enteric bacteria in the environment. In contrast, HICs generally have better infrastructure, including advanced water treatment facilities and effective waste management systems, which

help reduce contamination levels[26,27]. Additionally, people in HICs are more aware of hygiene practices, which helps reduce the spread of these bacteria[28]. However, even in HICs, potential outbreaks can occur due to emerging pathogens or aging infrastructure[25].

Role of WWTPs in reducing bacterial contamination

Wastewater treatment plants (WWTPs) play a critical role in reducing bacterial contamination in environmental water. The sewage water undergoes a series of treatment processes, including primary, secondary, and tertiary treatments, which physically, biologically, and chemically remove contaminants[29]. Many studies have shown the impact of WWTPs in reducing pathogenic bacteria. For instance, Bonetta et al.[30], in a study conducted in Italy, demonstrated a significant reduction in *E. coli* levels after the water was passed through WWTPs equipped with membrane bioreactor systems (MBS). Federigi et al.[31] demonstrated the effectiveness of tertiary WWTP processes, including UV radiation and chlorine dioxide disinfection, in further reducing *E. coli* contamination. Additionally, studies by Li et al.[32] and Azuma et al.[33] reported varying ESBL-Ec removal efficiencies, ranging from 51% in China to 99.9% in Japan. Moreover, Cheng et al.[34], in a study conducted in Ireland, highlighted the role of WWTPs in minimizing the contamination levels of *E. faecium* and *E. faecalis*. These differences may be attributed to variations in wastewater treatment technologies, environmental conditions, and monitoring methods used in different countries

Pharmaceutical pollution in environmental waters

Pharmaceuticals, including non-steroidal anti-inflammatory drugs (NSAIDs) and antibiotics, were previously reported to be the most commonly released substances in Swedish waters[35]. A previous study at Örebro University demonstrated that unknown substances in inland waters increased the inflammatory responses in exposed human cells[36]. Releasing antimicrobial drugs into environmental waters is potentially the leading cause of the emergence of antibiotic-resistant bacteria. Enterococci pose a potential health risk due to their ability to acquire antibiotic-resistant genes (ARGs) from other organisms in the environment[37,38]. It has been reported that there have been increasing numbers of antimicrobial-resistant enterococci and *E. coli* in the last few years[39,40]. Additionally, enteric bacteria are distributed to the environmental waters through human sewage outlets or animal wastes[40,41].

Consequently, epidemiological studies have also found a direct relationship between increasing cases of swimmers' gastroenteritis and high numbers of enteric bacteria, such as *E. coli* and enterococci, in surface waters[19,42,43].

Antibiotics and mechanisms of antibiotic resistance

"Antibiotics are substances that either kill bacteria or inhibit their growth"[44]. Their activities can be classified into two categories: bactericidal, which refers to antibiotics that actively kill bacteria, and bacteriostatic, which inhibit bacterial reproduction without directly killing the organisms. The new definition for bactericidal is one for which the ratio of the minimum bactericidal concentration (MBC) to the minimum inhibitory concentration (MIC) is ≤ 4, such as beta-lactams. In contrast, bacteriostatic agents have an MBC-to-MIC ratio of > 4, such as tetracyclines and macrolides[45,46]. Antibiotics that target the bacterial cell wall, such as β-lactams (e.g. penicillins, cephalosporins, carbapenems, and monobactams)[47] or interfere with essential enzymes, such as quinolones, are mainly bactericidal. while the antibiotics targeting protein synthesis are mostly bacteriostatic, for instance, tetracyclines, lincosamides, and chloramphenicol[48]. Furthermore, antibiotics can also be classified according to their antimicrobial spectrum into narrow-spectrum, such as penicillin, which acts only on Gram-positive bacteria[49,50], or broad-spectrum, such as meropenem, which can act on Gram-positive and Gram-negative bacteria[50]. Antibiotics are widely used in human and veterinary medicine, and in some countries, they are also used in animal husbandry as feed additives to promote animal growth[51]. Misuse or abuse of antibiotics is the primary reason for the emergence of antibiotic resistance in bacteria. Antibiotics are excreted by humans and animals primarily due to incomplete intestinal absorption, resulting in elimination through feces or as active metabolites that are excreted via urine and subsequently discharged into the sewage. Thus, human and animal waste contribute to an increase in the distribution of antibiotics in the environment and an increase in antibiotic-resistant bacteria[51,52].

Bacteria use different mechanisms to become resistant to antibiotics. One mechanism involves inactivating the antibiotic by hydrolysis. For example, β-lactamase enzymes found in *E. coli* can hydrolyze most β-lactam antibiotics, including penicillins and cephalosporins. The development of new generation β-lactam antibiotics has led to the emergence of resistant bacteria carrying extended-spectrum β-lactamases, which can hydrolyse third-generation cephalosporins (ceftazidime and cefotaxime). These enzymes are found in

some Enterobacteriaceae, for example, in *Klebsiella pneumoniae* and *E. coli*[53,54].

Adenylation or phosphorylation of the antibiotic is another inactivation mechanism utilized by bacteria, for instance, during the development of aminoglycoside resistance in *E. faecalis* and *E. faecium*[55].

Modification of the antibiotic target is another mechanism by which bacteria develop resistance. This results in reduced efficiency of the antibiotic's activity. For example, penicillins, cephalosporins, and vancomycin have reduced activity in bacteria that have altered their cell wall structure. The *vanA* gene in *E. faecium* alters the structure of peptidoglycan in the cell wall by encoding D-alanyl-D-alanine ligase, which participates in peptidoglycan formation, resulting in resistance to vancomycin[56].

Removal of the antibiotic from the bacterial cell through efflux pumps present in many bacteria can also result in antibiotic resistance. Efflux pumps are transmembrane proteins found in the bacterial cell wall that can export antibiotics from bacteria before they reach their target site. Generally, most efflux pumps are multidrug transporters, being capable of exporting a wide range of unrelated antibiotics such as macrolides and tetracyclines. Thus, this mechanism could contribute to multidrug resistance (MDR) in bacteria[57]. However, some of the efflux pumps vary in specificity for the substances they transport out of the cell. For instance, some *E. faecalis* strains encode for an efflux pump *emeA* (enterococcal multidrug resistance efflux) that resists a wide range of substances, including norfloxacin, ethidium bromide, and erythromycin[55,58]. A specific efflux pump gene known *msrC* was exclusively found in Enterococci, which confers resistance to macrolides[59].

Antibiotic-susceptible bacteria can become resistant due to the selection of de novo occurring mutations[60], which occur due to DNA replication errors or incorrect DNA repair. Resistance to quinolones in *E. coli* could be caused by the occurrence of at least seven different single-nucleotide changes in the target gene *gyrA*[57]. On the other hand, the acquisition of new genetic elements through horizontal gene transfer (HGT) is another mechanism by which bacteria become resistant. In this mechanism, the acquisition of new genetic elements can be through transduction, involving bacteriophages and integrons, or conjugation, transferring plasmids and conjugative transposons. Furthermore, the transformation of exogenous genetic elements released from dead bacteria is another mechanism for the acquisition of ARGs[60] (Figure 1).

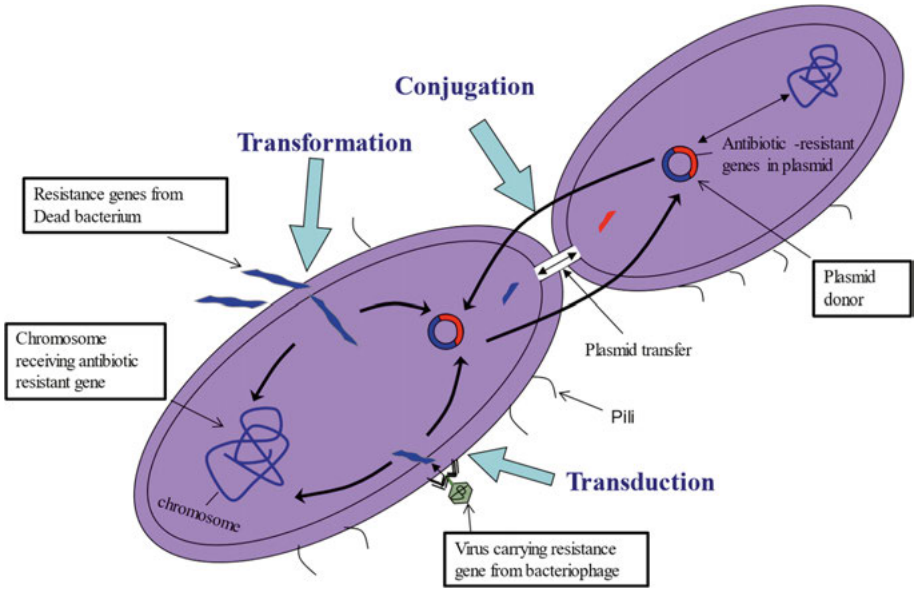

Figure 1. Illustration of the three main mechanisms for HGT of resistance genes in bacteria. Conjugation involves the connection of one bacterium to another via sex pili, facilitating the transfer of plasmids or genetic material that contains antibiotic-resistance genes (ARGs). Transduction involves transferring ARGs carried by a DNA virus from one bacterium to another (viral transduction). Transformation, as another mechanism of HGT, consists of a bacterium taking up ARGs that are present in its surroundings due to the death of a bacterium that contains ARGs.

β-Lactamase classifications and the clinical impact of ESBL.

The Ambler classification is the most widely used method for β-lactamases, categorizing them based on their amino acid sequences into four classes. These classes are: Class A (serine β-lactamases), such as CTX-M, TEM, and SHV enzymes; Class B (Metallo-β-lactamases (MBLs)), which require zinc ions for activity; Class C (AmpC β-lactamases), including CMY and DHA enzymes, which are not inhibited by clavulanic acid; and Class D (OXA-type β-lactamases), another group of serine β-lactamases[61,62]. ESBLs confer resistance to a broad range of β-lactam antibiotics and are primarily classified within Ambler Class A, which includes the CTX-M family and certain variants of the TEM and SHV enzymes[63]. These enzymes are produced by bacteria capable of hydrolyzing extended-spectrum cephalosporins and penicillins, making these bacteria resistant to many clinically relevant β-

lactam antibiotics[64]. ESBLs were first detected in the mid-1980s in *K. pneumoniae* isolates from intensive care unit patients in France[65]. Furthermore, developing resistance to third-generation cephalosporins has become a major concern in clinical settings, complicating infection treatment[66,67]. Initially, these resistant strains were predominantly identified in clinical settings, but they have since spread into the community. This increases the risk for urinary tract infections and other serious infections caused by ESBL-producing bacteria. Endogenous ESBL-Ec have been implicated as the primary driver of ESBL-Ec infections, and thus increased community carriage is likely to increase the rate of infections[68]. A study was conducted to estimate the burden of ESBL-Ec infection among European countries in 2015, which indicates that the median case numbers of infection caused by ESBL-Ec were 300,000 and 9,000 attributable deaths in that year[69]. Another study in Thailand found a mortality rate of 30.6% in patients with ESBL-producing *E. coli* bloodstream infections (BSI) compared to 22.2% in non-ESBL cases, highlighting the increased risk of mortality associated with ESBL-Ec in emergency department settings[70]. This resistance poses a major challenge for healthcare providers, emphasizing the urgent need for new therapeutic strategies and effective antimicrobial stewardship programs

Common ESBLs found in *E. coli*

The most common ESBLs reported in *E. coli* are CTX-M enzymes, which are classified into several groups based on their amino acid sequences, such as CTX-M-1, CTX-M-2, CTX-M-8, with CTX-M-15 and CTX-M-55 being particularly widespread[71-73]. Each group has minor allelic variants that differ by one or a few amino acid substitutions[71]. CTX-M-15 is the most prevalent variant found globally in clinically relevant settings, predominantly in human clinical isolates, and is a major contributor to antibiotic resistance in healthcare environments[74]. Notably, CTX-M-15 has also been detected in environmental sources, such as gulls, potentially due to contamination from human waste. Atterby et al.[75], in a study on Swedish gulls, demonstrated the presence of CTX-M-15 producing *E. coli* in freshly collected fecal samples, highlighting the potential dissemination of the ESBL genes between humans and gulls. Additionally, CTX-M-15 is strongly associated with the globally disseminated ST131 clone, which likely contributes to its rapid spread and persistence[76-78]. On the other hand, CTX-M-55, while also found in clinical settings, is more frequently associated with animal sources and environmental samples. For instance, Yang et al.[79], reported CTX-M-55-positive *E. coli*

strains isolated from cattle feces in China. It has also been reported in various environments, including food-producing animals, highlighting its role in the transmission of resistance genes between animals and humans[80]. Additionally, Yang et al.[73] also suggested that CTX-M-55-positive *E. coli* is frequently clonally transmitted between three human-animal environments: farms and agriculture settings, veterinary clinics, and households (human-animal interaction). This underscores its zoonotic transmission potential and growing importance in public health. In addition to CTX-M-15 and CTX-M-55, other notable CTX-M variants include CTX-M-27, which has recently emerged as a highly prevalent ESBL type, particularly in Europe and Asia. A study conducted in the Czech Republic found that 36.7% of ESBL-Ec isolates from patients with urinary tract infections were positive for the CTX-M-27 enzyme[81]. These enzymes are often carried on plasmids, which facilitate their rapid spread among bacterial populations, contributing to the persistence and dissemination of ESBL-Ec in both clinical and community environments[74,80].

Plasmids facilitating the spread of ESBL-Ec

Plasmids are a major vehicle of HGT in bacteria, playing a crucial role in the dissemination of ARGs across clinical, environmental, and agricultural settings[82]. They facilitate the transfer of resistance genes between different bacterial clones and species, which is essential for mobilizing naturally occurring resistance mechanisms and integrating them into clinical strains[83]. This process enhances the survival and persistence of resistant bacteria in diverse environments. In clinical settings, plasmids significantly contribute to the propagation of antibiotic resistance, making them key targets for molecular surveillance and control efforts[84]. Within the One Health framework, plasmids are vital due to their ability to transfer resistance genes across human, animal, and environmental domains[85]. This zoonotic transmission potential underscores the importance of monitoring plasmid-mediated resistance in both human and veterinary medicine[86]. Several types of plasmids result in the emergence of ESBL-Ec, including IncF, IncI1, IncN, and IncA/C[87]. Among these, IncF plasmids are the most prevalent and play a crucial role in disseminating ESBL genes[87]. IncF plasmids are highly adaptable and capable of transferring resistance genes between different bacterial strains[87,88]. They often carry multiple resistance genes, including those encoding for ESBLs such as $bla_{CTX\text{-}M}$, and bla_{SHV}[89]. Additionally, the high transfer frequency and stability of IncF plasmids contribute to their dominance among ESBL-Ec, making them a primary target for monitoring and controlling the spread of antibiotic resistance[87].

Multidrug-resistant bacteria

The literal definition of Multidrug-resistant (MDR) bacteria is "bacteria that are resistant to more than one class of antimicrobial agents". However, no standardized definition has been made yet. The latest proposed definition of MDR is "non-susceptibility to at least one agent in three or more antimicrobial categories"[90]. The accumulation of several different ARGs on mobile genetic elements (MGEs) is a primary factor in the development of most clinically important MDR phenotypes[91]. The risk of ARGs spreading between bacteria on plasmids and transposons is significant, as has been shown for the transfer of ARGs from *Shigella* to *E. coli*[92]. Additionally, plasmids can carry a gene encoding a specific enzyme that confers resistance to various antibiotic categories. For instance, bla_{NDM-1} is found in *E. coli,* which provides resistance to a broad spectrum of beta-lactam antibiotics, including carbapenems, cephalosporins, and penicillins[93]. The *erm(B)* is another gene found in many bacterial species, where mutations have been shown to confer MDR. Thus, mutations in this gene result in resistance to macrolides, lincosamides, and streptogramin B antibiotics[94-96]. Co-occurrences of ARGs on plasmids also play a significant role in MDR phenotypes. For instance, plasmids carrying both bla_{CTX-M} (β-lactam resistance) and *qnr* genes (quinolone resistance) are prevalent in clinical *E. coli* isolates[97,98]. Similarly, plasmids with bla_{NDM} (carbapenem resistance) and *mcr* genes (colistin resistance) have been found in *E. coli*[99]. These co-occurrences increase the potential for resistance dissemination and highlight the importance of monitoring plasmid-mediated resistance in both clinical and community settings[100]. Plasmids can carry efflux pump genes, which may help expel various antibiotics that enter the bacterial cell, thereby contributing to the MDR phenotype. For example, the overexpression of the *acrB* gene in *E. coli* potentially ejects different types of antimicrobial agents, including β-lactams, fluoroquinolones, tetracycline, chloramphenicol, and acriflavine[56]. Furthermore, a study demonstrates that the overexpression of *AcrAB* efflux pump in *K. pneumoniae* plays a crucial role in mediating resistance to tetracyclines and chloramphenicol[101]. While overexpression of these pumps can enhance resistance, it does not necessarily result in high-level resistance on its own[102].

Impact of antibiotic resistance genes on pathogenic bacteria and healthcare costs

Antibiotic resistance in pathogenic bacteria, mainly due to the presence of ARGs, contributes significantly to treatment failure, prolonged hospital stays,

and increased mortality rates[21]. The accumulation of these genes on plasmids, which facilitate HGT, plays a crucial role in the development of MDR strains such as ESBL-producing Enterobacteriaceae, making them difficult to treat with standard antibiotics[91,103]. ESBL-Ec has emerged as a significant cause of healthcare-associated infections worldwide[104]. Tumbarello et al.[105] reported that ESBL production increased the average hospital stay following the onset of BSI by approximately 7 days and raised hospital costs by about €5,026. Additionally, in LMICs, where healthcare resources are limited, the financial burden is even more pronounced due to inadequate infection control measures and, in some cases, the use of expired antibiotics[106]. For instance, Bayaba et al.[107], in a study conducted in Cameroon, indicated that 82% of ESBL-*Enterobacterales* were MDR, with high resistance rates to amoxicillin-clavulanate (81.1%) and ceftazidime (67.56%). Moreover, another study conducted in Indonesia found that the mortality rate for patients with ESBL-Ec BSI was 30.6%, compared to 22.2% for non-ESBL cases[108]. These findings highlight the clinical burden posed by enteric bacteria carrying ARGs, emphasizing the need for continuous surveillance programs to mitigate the economic impact of antibiotic resistance. Addressing the challenges posed by antibiotic resistance requires innovative strategies beyond traditional antibiotics, such as bacteriophage therapy and antimicrobial peptides, both of which have recently shown promise in combating resistant infections[109,110]. Additionally, understanding the dynamics of ARG dissemination can aid in developing new antibiotics targeting MDR pathogens while minimizing healthcare costs associated with resistance.

Host specificity and genetic relatedness of *E. coli*

E. coli is a very diverse species of bacteria that can inhabit different habitats, like humans, animals, and the environment. Insights about how *E. coli* adapts to different hosts and its genetic diversity are important for understanding how it spreads and causes disease[111]. Host specificity is observed in certain isolates of *E. coli* due to factors such as adhesins and toxins, which help bacteria stick to and infect the host. For example, some *E. coli* strains are adapted to the guts of humans and animals, while others are found more in the environment[112].

The genetic diversity of *E. coli* is very high due to gene transfer events, mutations, and recombination. Despite this, studies show that *E. coli* strains from different hosts and environments can have unique genetic profiles, with strains being categorized into phylogenetic groups based on their genetic relatedness. These can be studied using methods like Clermont typing, Multi-Locus Sequence Typing (MLST), and core genome MLST (cgMLST)[113,114].

Some *E. coli* clones are observed in both humans and livestock globally, with varying degrees of human-animal transmission. For example, ST131 is commonly recorded in clinical settings[115], while ST23 is often found in livestock[116]. On the other hand, common STs like ST10 and ST69 are likely shared between humans and livestock[117]. This global distribution shows how adaptable *E. coli* is to different hosts and environments.

There is strong evidence that *E. coli* can be transmitted between humans and livestock, especially when they are in close contact or shared environments. Studies have found that *E. coli* strains from humans and livestock in the same area can be genetically identical, showing high rates of gene flow between these hosts[118-120]. This transmission can spread AMR and zoonotic infections. Phylogenetic analysis helps us understand the evolutionary relationships and host adaptation of *E. coli* strains. By comparing genetic sequences, researchers can identify clonal lineages linked to specific hosts and track the spread of pathogenic strains. This is crucial for understanding *E. coli* infections and developing targeted interventions[121].

Implications for public health and policy

The rise of antibiotic-resistant enteric bacteria is a significant problem for public health. Due to limited treatment options, these bacteria can cause more severe and prolonged illnesses, leading to higher morbidity and mortality rates. This is primarily because treatments are less effective, and resistance can co-occur with increased pathogenicity in some strains[107] or because plasmids can simultaneously transmit antibiotic resistance and pathogenicity-related genes[40]. The economic burden on healthcare systems is also significant, with increased costs resulting from longer hospital stays, additional diagnostic tests, and more expensive drugs[21]. Addressing this issue requires a comprehensive approach that considers the interconnectedness of human, animal, and environmental health.

One critical approach is to improve waste management, particularly WWTP, to reduce the spread of antibiotic-resistant bacteria in the environment[30]. This helps to limit the contamination of water sources and the broader ecosystem, which can be a reservoir for resistant bacteria. Improving sanitation, such as a clean water supply, can reduce the transmission of resistant bacteria[122].

Another key strategy is to reduce and optimize antibiotic use in both humans and animals. This includes implementing antibiotic stewardship programs in healthcare settings to ensure appropriate prescribing, adjusting dosages and treatment durations based on clinical evidence, and minimizing

unnecessary use. These efforts help slow the emergence of resistance while maintaining treatment effectiveness. In parallel, infection prevention and control measures, such as hand hygiene and environmental cleaning, are essential to prevent the spread of resistant bacteria[122,123].

Enhanced surveillance is also essential for informing public health policy. By increasing surveillance in humans, animals, and their surrounding environment, especially in LMICs, we can better understand the scope of the problem and customize interventions accordingly. Surveillance data can inform public health strategies and help identify emerging resistance patterns.

Lastly, adopting the One Health approach in health policies is crucial. This approach recognizes the interconnectedness between human, animal, and environmental health[124]. Policies should promote teamwork across different sectors to address health threats. By setting up systems to monitor antibiotic resistance in humans and livestock, we can respond to new threats quickly. Educating policymakers, healthcare providers, and the public about One Health can lead to a more coordinated and effective response to antibiotic resistance.

Aims:

The general aim of the thesis is to enhance understanding of the abundance and antibiotic resistance characteristics of enteric bacteria from a One Health perspective.

Specific Aims:

- Paper I: Describe the distribution of, and prevalence of antibiotic resistance among, *Enterococcus faecalis, Enterococcus faecium*, and *E. coli* in surface waters upstream and downstream of the WWTP in Örebro, Sweden.

- Paper II: Characterize and describe spatiotemporal variation of abundance, phylogeny, and antibiotic resistance profiles of 3GCR-Ec, including ESBL-Ec, in surface waters upstream and downstream of the WWTP in Uppsala, Sweden.

- Paper III: Analyze genotypic antibiotic resistance profiles and genetic relatedness to infer geographic and host-related transmission patterns among ESC-Ec from humans and livestock in rural Cambodia.

Methods

Study designs and sampling

Most of the studies included in this thesis (Papers I and II) were conducted in two Swedish rivers. The first study (Paper I) was carried out at Svartån (59.2747° N, 15.2151° E), in Örebro city, located in the middle of the country (Fig. 2A). Paper II was conducted at Fyrisån (59.7833° N, 17.6500° E), in Uppsala city, located 71 km north of Stockholm (Fig 2B). Paper III was conducted in Kampong Cham, Cambodia (11.9934° N, 105.4635° E) (Fig. 2C). Water samples were collected from Svartån and Lake Hjälmaren area near Örebro city at the following locations: Svartån at Tekniska Kvarn, Svartån at Naturens Hus, Hemfjärden, Mellanfjärden and Storhjälmaren, respectively (Fig. 2A). The water samples were also collected from Fyrisån in Uppsala city at the following locations: Upstream of the WWTP (upWWTP), near the effluent of WWTP, downstream of the WWTP (dWWTP), and the recipient river (Lake Målaren) (Fig. 2B). The samples were collected in Örebro during the summer, and autumn of 2011 and 2012, while the samples from Uppsala were collected during March, May, July, September, and November of 2023. To reduce the risk of false-positive CFU counts resulting from surface debris or airborne contamination, sterile bottles were submerged approximately 50 cm below the water surface. The water samples were kept at 4-6˚C and filtered within 24 hours for microbiological analysis. In Paper III, Fecal bacteria from humans and animals in Kampong Cham, Cambodia, were collected during a previous study conducted by our lab in May 2011[125,126]. The isolated ESBL- and/or AmpC-producing *E. coli* strains were then subjected to in-silico analysis.

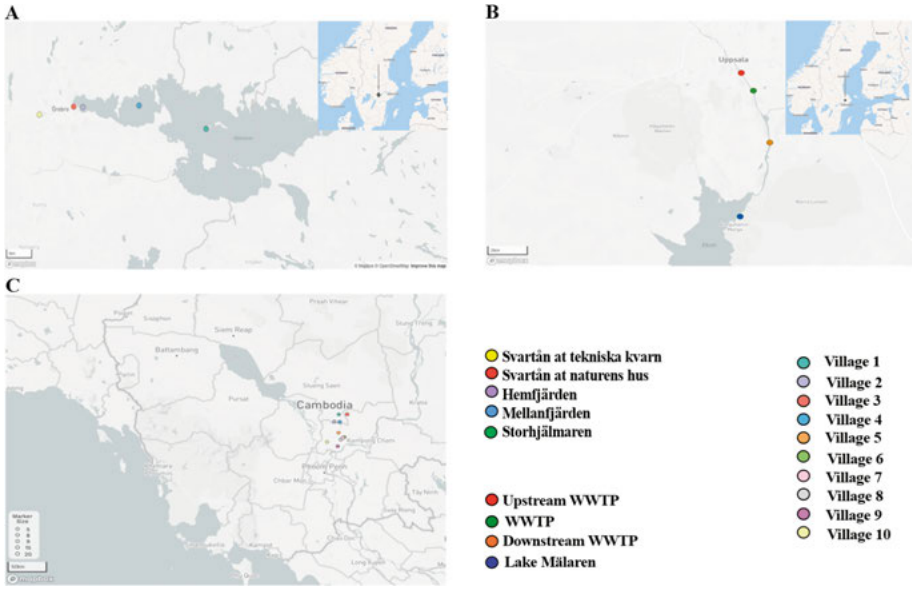

Figure 2. **(A)** Map showing Örebro, Sweden location (grey circle) with a zoom-in of the study locations of Svartån River through Örebro city into Lake Hjälmaren, highlighted with different colors. Sample sites were Svartån River at Tekniska Kvarn, upstream of the WWTP effluent (yellow circle), Svartån Naturens Hus, recipient site of the effluent from Örebro wastewater treatment plant (Red circle), Hemfjärden, the lake into which Svartån River empties and is adjacent to Oset bird sanctuary (violet circle), Mellanfjärden, the central part of Lake Hjälmaren (blue circle), and Storhjälmaren, the deepest, largest, and furthest east in the Lake (Green circle). **(B)** Map showing Uppsala, Sweden (grey circle) with a zoom-in of the sampling locations at Uppsala along the Fyrisån river: upstream and downstream of the WWTP, with recipient lake Mälaren highlighted in different colors. **(C)** Map of Cambodia showing the sampling village locations for the ESBL- and AmpC-producing *E. coli* strains from humans and livestock (V1–V10) highlighted by different colors. **All maps are generated using microreact and visualized using Inkscape software version 1.4.**

Microbiology

Water samples from Papers I and II were filtered through nitrocellulose filters with a pore size of 0.45 µm, using filters from Sartorius Stedim (Sweden) and Cytiva (Japan), respectively. The membrane filters were then incubated either on membrane-filter *Enterococcus* selective medium (Merck KGaA, Darmstadt, Germany) for 36 to 48 hours at 37°C or on *E. coli* specific CHROMagar™ C3GR medium (CHROMagar, Paris, France) for 24 hours at 37°C. Enterococci and 3GCR-Ec colonies were counted and reported as CFU per 100 ml for each location (**fig. 3 A and B**). Random single colonies with characteristic coloration-red for Enterococci and pink for *E. coli* were isolated from the filter papers and grown on brain heart infusion (BHI) (Difco™, BD

Diagnostics, Sparks, MD, USA) and CHROMagar™ C3GR, respectively. The Enterococci and 3GCR-Ec isolates were stored in BHI and Lysogeny Broth (LB) broth (Thermofisher, Kandel, Germany), respectively, with 15% glycerol at -80˚C for further analysis. In Paper III, the microbiological analysis of ESC-Ec obtained from Kampong Cham, Cambodia, was conducted in a previous study by our lab[126].

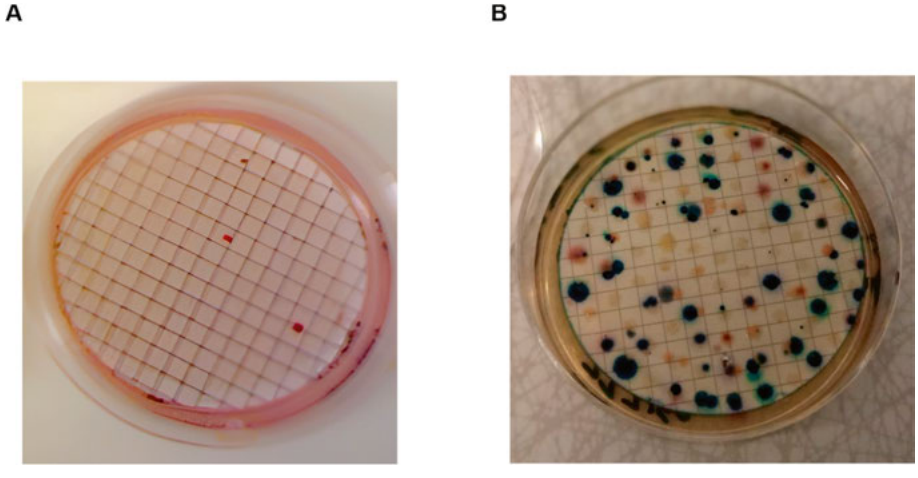

Figure 3. **(A)** A Petri dish plate showing membrane-filter Enterococcus selective media for water samples. Nitrocellulose filters with a pore size of 0.45µm were used to filter the enterococci water samples per 100 ml. Enterococci colonies are colored red. **(B)** A plate showing CHROMagarTM C3GR medium. Nitrocellulose filters with a pore size of 0.45µm were used to filter the coliform water samples per 100 ml. The pink colonies were identified as presumptive *E. coli,* while the blue and yellow colonies were other coliforms

Bacterial DNA extraction

After obtaining pure bacterial colonies of *E. coli*, *E. faecium*, and *E. faecalis*, genomic DNA (gDNA) was extracted using a modified boiling method described by Bollet et al[127]. Briefly, four to six pure single colonies were suspended in 200 µL of sterile distilled water in a 1.5 mL Eppendorf tube. The suspension was then microwaved at 650 W for 4 minutes for *E. coli* and 6 minutes for Enterococci in a water bath. The bacterial suspension was centrifuged at 13,330 rpm for 2 minutes using a MICRO-STAR 17 centrifuge (VWR, Belgium). The supernatant was transferred into a new 1.5 ml sterile Eppendorf tube and saved at -20℃. The gDNA of enterococcal isolates was used for polymerase chain reaction (PCR) if the concentration was >70 ng and the quality of protein contamination (A260/A280) ratio ranged between 1.80 - 2.00.

In Paper II, whole-genome sequencing (WGS) of *E. coli* isolates was performed at Novogene using the Illumina platform when the concentration of genomic DNA was above 100 ng/µl, and the A260/A280 ratio was 1.8-2.0. The concentrations of extracted DNA were measured using a Qubit fluorometer (Thermo Fisher Scientific, USA) and assessed for protein contamination using Nanodrop (Thermo Scientific NanoDrop 1000 UV-VIS Spectrophotometer, USA).

The gDNA isolation of ESC-Ec isolates obtained from Cambodia (paper III) was performed in a previous study by our lab[126].

Molecular analysis

Genotypic identification of enterococcal isolates was done by performing multiplex PCR. The presence of antibiotic resistance genes was determined by performing a conventional PCR. The PCR was performed using a Dream taq ^{TM} polymerase Kit (Fermentas, Sweden) with specific primers (Paper I - Tables S2-3). Specifically, the PCR was performed using 70ng-300ng of template gDNA in a 25 µl reaction mixture containing 1 x PCR Dream Taq buffer, 0.2 mM dNTPs, 0.6 mM of primers for *ddl*, 0.1 mM of primers for *16srRNA,* and 0.625 U Taq polymerase. The PCR reaction conditions consisted of one cycle of initial denaturing at 95˚C for 5 min, 30 cycles of denaturation at 95˚C for 30 sec, annealing at 53˚C for 45 sec, and an extension time was 1-2 min at 72˚C, and a final cycle of extension for 10 min at 72˚C. *E. faecium* strain ATCC 19434 and uropathogenic *E. faecalis* 1131 were positive controls in the PCR reactions. PCR products were loaded with DNA loading buffer into a 1% agarose gel containing 1% ethidium bromide. Electrophoresis was done in TAE (Tris-Acetate, EDTA pH 8) buffer for 30 min at 90 volts, and the expected product bands were visualized using a UV in agar (BioDoc, Cambridge, UK).

In Paper II, species identification of 3GCR-Ec isolates was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) at the Department of Clinical Microbiology, Uppsala University Hospital (BRUKER). WGS was performed on these isolates for further verification and genomic analysis.

Biochemical Identification Test

Enterococcal strains were cultured overnight on BHI at 37°C. A single colony was then sub-cultured on Columbia blood agar (Difco™, BD Diagnostics,

New Jersey, USA) at 37°C for 24 hrs. Analytic Profile Index (API/ID 32) for biochemical bacterial identification was performed according to the manufacturer's instructions (BioMérieux, Marcy-l'Étoile, France) to confirm species. Uropathogenic *E. faecalis* 1131 was selected as the quality control strain for API analysis.

Antibiotic susceptibility test

In paper I, the Enterococcal strains were tested for antibiotic sensitivity using the Epsilometer test (E-test). Selected enterococcal environmental isolates (*E. faecium* and *E. faecalis*) were grown overnight on BHI at 37˚C. Single colonies were taken from sub-cultured enterococcal growth and suspended in 0.9% saline, and the optical density (OD_{600nm}) of the inoculum was measured according to McFarland 0.5 standard. The inoculum was streaked onto Mueller Hinton (MH) agar (pH 7.3±1) in three directions, after which the appropriate antibiotic strips were placed on the plate. The MIC was determined for tetracycline, vancomycin, ampicillin, gentamycin, streptomycin, and imipenem. The selected antibiotic strips were applied to MH media in a 150 mm petri dish, and the inoculum was incubated for 36 to 48 hr at 37˚C. *E. faecalis* ATCC 29212 was used as the control for the antibiotic susceptibility assay.

In paper II, the selected 3GCR-Ec isolates (n = 40) were tested for their susceptibility to additional antibiotics using Sensititre™ GNX3F plates (Thermo Fisher Scientific, West Sussex, UK), following the manufacturer's instructions. Briefly, two to three overnight pure single colonies were suspended in 5 mL of sterile saline (0.9 %). The suspension was adjusted to McFarland 0.5 by measuring the (OD_{600nm}) using a Sensititre™ Nephelometer (Thermo Fisher Scientific, US). Fifty µl of adjusted inoculum was transferred into 10 ml of Muller-Hinton (MH) broth. Fifty µl of the working inoculum was distributed to each 96-well plate of GNX3F using a multi-pipette. The plates were incubated overnight at 37°C, and the results were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[128].

In-silico analysis

Genomic *E. coli* strains for Paper II (3GCR-Ec) and Paper III (ESC-Ec) were analyzed using various bioinformatic tools, as described below:

1- **Genomic assembly, MLST, cgMLST, and phylogroup classifications (Clermont Typing).**

In papers II and III, de novo assembly of our Illumina raw reads was performed using the Enterobase database tools[129]. The QAssembly pipeline v3.61, employed Sickle v1.33[130] for quality- and adapter-trimming[129]. The trimmed reads were assembled using SPAdes v3.9.1[131]. Reads were aligned back to the assemblies to improve consensus base call accuracy using BWA v0.7.12-r1039[132]. Kraken v0.10.5-beta[129,133] was used to detect inter-species contamination, with a threshold of ≥70% of contigs assigned to *E. coli*. A second verification of genome quality was performed with CheckM v1.1.3[129,134], ensuring completeness above 99% and a contamination value of less than 1% (Paper II, Table S2) and (Paper III, Table S1). For strain typing, MLST was performed using the NServ genotyping tool integrated into EnteroBase. In Paper III, additional high-resolution typing was conducted using cgMLST, and Clermont Typing was applied to assign phylogroups.

2- **Pangenome and single-nucleotide polymorphism (SNP) tree construction.**

In Paper II, a pangenomic phylogenetic tree was constructed for 3GCR-Ec isolates based on core gene alignments. Genomes were annotated using Prokka v1.14.6[135], and annotations including the reference genome *E. coli* K-12 (NC_000913.3) were processed with PIRATE v1.0.5[136], as described before[137]. Briefly, Orthologous genes were clustered at various Markov Cluster algorithm (MCL) thresholds using default parameters. Core genes present in >95% of isolates were aligned using MAFFT. A maximum likelihood phylogenetic tree was generated using RAxML v8.2.11[138], with the substitution model GTRGAMMA.

In Paper III, a phylogenetic tree of ESC-Ec strains was constructed using core SNPs. Genomes were annotated using Prokka v1.11[135], and the assemblies were mapped to the reference genome of *E. coli* 169757 (NC: JAAKGF000000000.1) using refMapper v1.0[129]. SNPs were identified by aligning the assemblies to the reference genome with the LAST package v5.88[129]. SNPs with a quality score below 10 or alignment ambiguities above 0.1 were excluded. The filtered SNPs were combined into a matrix file using refMapperMatrix v2.0[129], and the resulting SNP matrix was used to implement

a maximum likelihood phylogenetic tree in RAxML v8.2.9[138,139], applying GTRGAMMA as the substitution model.

Microreact was used to visualize both the SNP and pangenome-based phylogenetic trees.

3- **Screening for ARGs and plasmids**

All selected *E. coli* genomes from Papers II and III were screened for the presence of ARGs using ResFinder v4.6.0[140,141]. For 3GCR-Ec isolates (Paper II), a positive ARG hit was defined as a sequence match with >90% identity over >70% of the gene length. For ESC-Ec isolates (Paper III), a positive hit was defined as a sequence match with >90% identity over >90% of the gene length. Additionally, plasmid replicons were identified only in Paper III using PlasmidFinder v2.1[141,142], with a positive hit defined as a sequence match with >90% identity over >70% of the sequence length.

Statistical analysis

The Shapiro–Wilk test was performed in Paper II to evaluate the normality of CFU data for 3GCR-Ec obtained from the Fyrisån River. A P-value > 0.05 was considered indicative of a normal distribution. The Student's t-test was used to compare the parametric CFU groups (WWTP and dWWTP), while non-parametric groups (upWWTP and Mälaren Lake) were analyzed using the Mann-Whitney test. These analyses were performed using Prism v10.4.0 for Windows 10, GraphPad Software, Boston, Massachusetts, USA. Differences were considered statistically significant at $P \leq 0.05$ and are indicated with asterisks * in the plots. Unless specified otherwise, summary statistics are presented as medians with interquartile ranges (IQR) in parentheses.

Results and discussion

Paper I. Multi-Drug Resistant Fecal Bacteria in a Swedish Lake System

This study aimed to investigate the distribution of fecal indicator bacteria (FIB), including those with MDR, in the inland waters of Örebro, Sweden. The focus was on the Svartån river, both upstream and downstream of the WWTP, as well as the recipient Lake Hjälmaren, including Hemfjärden, Mellanfjärden, and Storhjälmaren. During the summer and autumn seasons of 2010 and 2011, our focus was on screening for *E. faecalis, E. faecium,* and *E. coli,* which are among the most common enteric bacteria used for assessing water quality[143,144]. Additionally, we evaluated the sensitivity of these microbes to different types of antibiotics (displayed in Paper I, Table 1). Our results showed that the distribution of fecal indicator bacteria was highly concentrated in Svartån at Naturens Hus near the WWTP compared to the

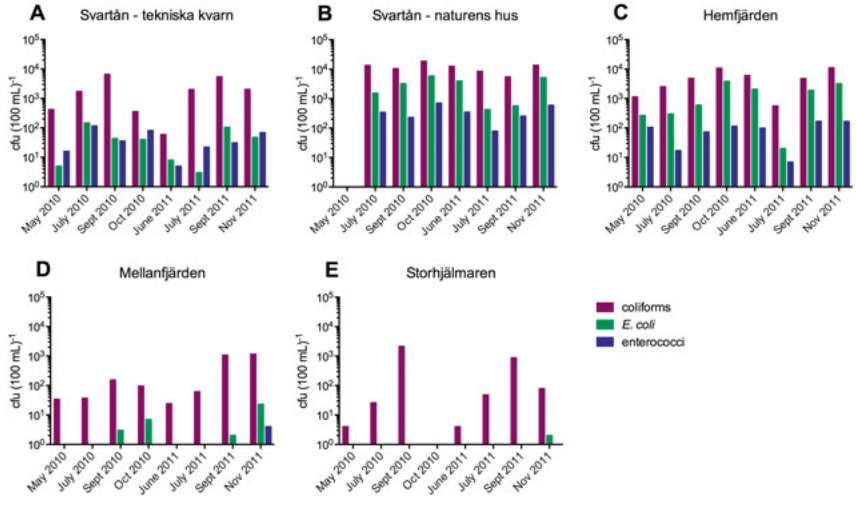

Figure 4. Figure 4. Spatiotemporal variations of fecal indicator bacteria in the Svartån River and Lake Hjälmaren during 2010 and 2011. Surface water samples were collected at four time points across both years and analyzed for total coliforms, *E. coli*, and enterococci. Sampling sites included: (A) Tekniska Kvarn (upstream of Örebro city), (B) Naturens Hus (near the WWTP effluent), and in Lake Hjälmaren at (C) Hemfjärden, (D) Mellanfjärden, and (E) Storhjälmaren. Note: samples were not collected at Naturens Hus in May 2010 and at Storhjälmaren in October 2010. Adapted from Figure 2 of Paper I**.**

other locations (Fig. 4A-E). While the lower CFU concentrations were detected in Mellanfjärden and Storhjälmaren, (Fig. 4D-E). This suggests that the WWTP likely contributes to the increased levels of FIB in Svartån at Naturens Hus, which aligns with findings by Cheng et al.[34] and Li et al.[32]. In 2010 and 2011, the prevalence of resistant *Enterococcus* strains (*E. faecium* and *E. faecalis*) was 12% (13 / 109) and 15% (16 / 110), respectively. For *E. coli*, the prevalence of resistant strains was 10% (11/ 110) in 2010 and 17% (20 / 120) in 2011. MDR bacteria were also identified among resistant *Enterococcus* spp. and *E. coli* in both study years (Table 1).

Table 1. Antibiotic resistance phenotypes of *E. coli* and enterococci from 2010 and 2011**.** This table is adapted from Table 4 of Paper I**.**

Bacteria	Antibiotic resistance phenotype [a]	Number of isolates	
		2010	2011
E. coli	SXT	1	1
	TET	2	7
	NAL	2	5
	TET-NAL	1	1
	TET-SXT	0	1
	TET-CHL	0	1
	TET-CHL-SXT	1	0
	TET-NAL-CHL	1	0
	TET-NAL-SXT	0	3
	TET-NAL-SXT-CIP	0	1
	TET-NAL-CHL-CIP	1	0
	NAL-CIP-CTX-CAZ	1[b]	0
	TET-NAL-CHL-SXT-CIP-CTX-CAZ	1[c]	0
Enterococcus spp.	TET	11	9
	VAN	0	1[d]
	STR	0	1
	TET-STR	0	1
	TET-AMP-GEN	1	0
	TET-STR-GEN	1	3
	TET-AMP-STR-IPM	0	1

[a] TET, tetracycline; CHL, chloramphenicol; NAL, nalidixic acid; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; CTX, cefotaxime; CAZ, ceftazidime; AMP, ampicillin; GEN, gentamicin; STR, streptomycin.

[b] AmpC overproducing isolate

[c] ESBL-producing isolate

[d] *vanA* gene

In 2010, 15% (2/13) of the enterococci resistant strains were MDR. By 2011, this percentage had risen to 31% (5/16) as detailed in Table 1. This finding is particularly concerning given that the study was conducted in a relatively unpolluted region of a high-income country with strict antibiotic regulations. The detection of MDR bacteria in such settings underscores a significant threat to public health and highlights the need for continuous environmental surveillance.

Tetracycline resistance was the most widespread antibiotic resistance observed in both FIB (Table 1). This finding was in line with previous studies[141,142], which reported tetracycline as the most commonly detected antibiotic resistance in environmental samples. Vancomycin is the drug of choice for enterococci resistant to penicillin or MRSA[145]. Previous studies reported that 18% of enterococcal isolates from WWTP in Sweden were vancomycin-resistant enterococci (VRE) in 2000[38], and approximately 22% of enterococcal isolates were VRE in urban treated waters in the UK[146]. In 2011, we found that one isolate among the resistant *Enterococcus* spp. (1 of 16; Table 1) was resistant to vancomycin. Resistance to β-lactams and production of extended-spectrum β-lactamases (ESBL) were confirmed in one resistant *E. coli* isolate out of 11 in 2010 (Table 1), using double disc synergy testing (DDST). This suggests that clinically relevant strains (ESBL-Ec and VRE) were released into environmental waters, potentially not removed by WWTP, or introduced through other sources such as recreational activities or migrating birds. This bacterial contamination potentially increases the risk to the exposed population. ARGs detection for the corresponding antibiotics tested in resistant *Enterococcus* isolates was performed using PCR. Previous investigations have identified the *tetM* gene as the predominant determinant of tetracycline resistance in *Enterococcus* spp[147-149]. In the present study, the detection of *tetM* in resistant *Enterococcus* isolates was consistent with these earlier findings. This suggests that *tetM* is potentially responsible for tetracycline resistance in the isolates examined (Table 1). On the other hand, we found some isolates show phenotypic resistance to certain antibiotics, even though they do not carry the corresponding ARGs. For instance, in 2011, one of the enterococcal resistant isolates was phenotypically resistant to ampicillin, while it did not carry *pbp5,* which mainly confers resistance to ampicillin[150,151]. Additionally, two out of three gentamicin-resistant enterococci harboured *aac6-aphII,* which commonly confers resistance to gentamicin (Table 2)[152,153].

Table 2. Genotypic confirmation of antibiotic-resistant fecal indicator bacteria in 2010 and 2011**.** This table is adapted from Table 3 of Paper I.

Bacteria	Antibiotic phenotype	Antibiotic-resistant gene	Genotypic n/ phenotypic n*	
			2010	2011
E. coli	chloramphenicol	*catI*	3/4	0/1
		catII	0/4	0/1
		cmlA	1/4	1/1
	nalidixic acid	*gyrA*	6/7	10/10
		gyrB	7/7	10/10
		parC	7/7	10/10
	tetracycline	*tetA*	1/7	9/14
		tetB	4/7	4/14
		tetC	0/7	0/14
		tetD	1/7	1/14
Enterococcus	ampicillin	*pbp5*	1/1	0/1
	gentamicin	*aac6-aphII*	2/2	2/3
	streptomycin	*aadE*	1/1	4/6
	tetracycline	*tetL*	0/13	0/14
		tetM	13/13	14/14
	vancomycin	*vanA*	0/0	1/1
		vanB	0/0	0/1

*Number of phenotypically resistant isolates carrying the corresponding resistance gene out of the total number of phenotypically resistant isolates.

This could be due to potential activation of repressor genes[154], other corresponding resistant genes not screened in this study, or an efflux pump gene[95,155,156]. Therefore, integrating phenotypic and genotypic tests, such as PCR targeting known ARGs, is crucial for understanding bacterial resistance. However, incorporating WGS in future studies would provide deeper insight into the full resistome, including resistance genes not screened in this study.

We conclude that the distribution of FIB was high in Svartån at Naturens Hus near the WWTP effluent compared to the other locations. Furthermore, the presence of ESBL-Ec and VRE, along with other MDR strains, underscores the need to improve the performance of the WWTP to limit the dissemination of enteric bacteria into the aquatic environment.

Paper II. Temporal and spatial variation of third-generation cephalosporin-resistant and ESBL-producing *Escherichia coli* in a Swedish urban water system

This study aimed to characterize the abundance of 3GCR-Ec isolates in the surface water of Fyrisån River in Uppsala, Sweden (Fig. 5A). The CFU averages of 3GCR-Ec, including ESBL-Ec isolates, were measured at different times and locations. For a comprehensive understanding of resistance profiles, antibiotic susceptibility and WGS analysis were performed on selected isolates (n=40) of 3GCR-Ec. Our results showed a significantly higher abundance of 3GCR-Ec, including ESBL-Ec, immediately downstream of the WWTP compared to Lake Mälaren ($P < 0.05$). Additionally, higher CFU concentration of 3GCR-Ec was observed at the WWTP compared to the upWWTP ($P < 0.05$), with a trend towards higher ESBL-Ec concentrations ($P = 0.0556$) (Fig. 5B-E). The finding highlights the potential impact of WWTPs in contaminating environmental water with antibiotic-resistant enteric bacteria, in line with previous studies in China and Japan[32,33]. On the other hand, we observed lower levels of CFU at dWWTP compared to WWTP, but the difference failed to reach statistical significance. This may be partly due to the relatively small sample size, which could have limited the statistical power to detect a true difference. Additionally, ecological factors such as the presence of migratory birds downstream of WWTP may contribute to the observed patterns. Migratory birds are known to carry and disseminate antibiotic-resistant bacteria over long distances, potentially introducing resistant strains into the river ecosystem[157]. Fluctuations in the average CFUs at different time points were observed (Figure 5B and 5D). The highest CFU concentration for 3GCR-Ec was found in November, while the lowest CFU was detected in July (Fig. 5B and 5D). These fluctuations in CFU concentrations could be due to several factors. In July, increased potential solar UV radiation may reduce bacterial survival in surface waters. Additionally, the reduced local human activity due to vacations likely results in lower contamination levels. In contrast, November sees the return of residents and increased wastewater discharge, and reduced sunlight exposure, which may enhance bacterial survival. A study indicated that the effect of solar UV may reduce bacterial growth in the aquatic environment[158].

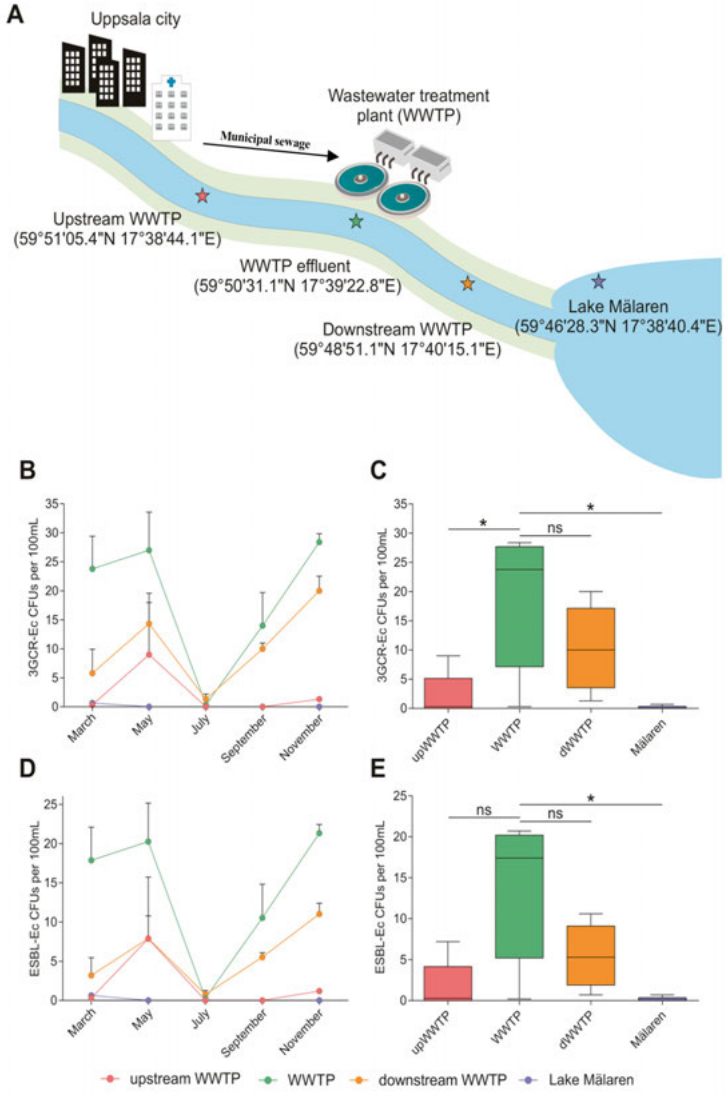

Figure 5. **Overview of sampling locations and variation in 3GCR-Ec and ESBL-Ec concentrations in the Fyrisån River, Uppsala**. (A) Schematic map showing four sampling sites along the Fyisån river: upstream of the WWTP (upWWTP, red star), near the WWTP effluent (WWTP, green star), downstream of the WWTP (dWWTP, orange star), and the recipient Lake Mälaren (blue star). (B) Temporal variations in 3GCR-Ec levels (CFU/100 mL) across sampling points.(red = upWWTP, green = WWTP, orange = dWWTP, blue = Mälaren). Error bars represent the standard error of the mean (SEM). (C) Box plots of 3GCR-Ec concentrations by site. (D) Temporal variation in ESBL-Ec concentrations. (E) Box plots of ESBL-Ec concentrations by site. Pairwise comparisons were performed using Mann-Whitney U tests; significance ($P < 0.05$) is indicated by an asterisk (*), and non-significance by "ns." The center line represents the median value, while the upper and lower bounds of the boxes correspond to the first and third quartiles. Adapted from Figure 1 of Paper II.

To further understand the characteristics of our 3GCR-Ec isolates, we assessed their susceptibility to twenty-one antibiotics (Paper II, Table S4). All tested isolates were MDR, consistent with findings from a study by Adekanmbi et al.[159] in Ibadan, Nigeria, where 3GCR-Ec isolates from river water were also MDR, underscoring the broad spectrum of antibiotic resistance observed in these bacteria.

The number of antibiotics to which the isolates were resistant was fairly consistent over time and across locations (Fig. 6A-B). On average, resistance towards different antibiotics was highest in samples from the upWWTP in May (12 antibiotics), while it was the lowest number in samples from the WWTP in September (5 antibiotics) (Fig. 6A). The slight variation in the number of antibiotics found in isolates is potentially influenced by the predominant water sources at different times that pass through the WWTP, which may affect the level of antibiotic residues.

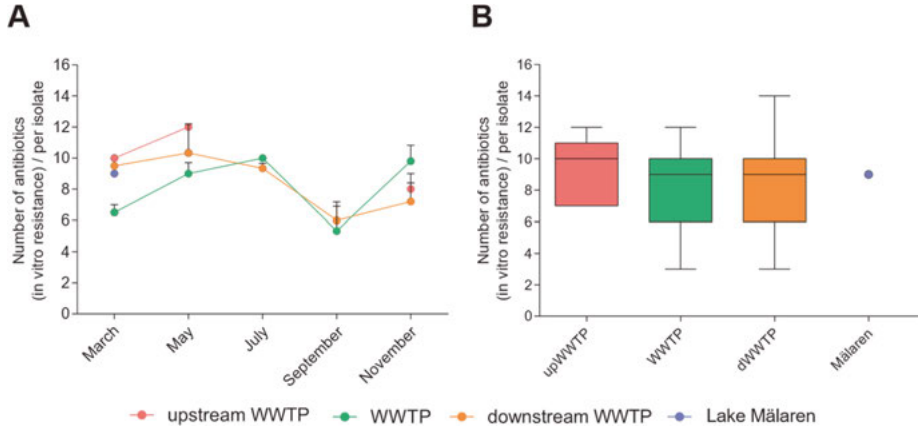

Figure 6. **Variation in the number of antibiotic resistances among 3GCR-Ec isolates across sampling sites and time points**. (A) Temporal variation in the number of antibiotics each isolate was resistant to, based on in vitro antimicrobial susceptibility testing (AST). (B) Box plots showing the number of antibiotic-resistant resistant per isolate at sampling site. Error bars represent the standard error of the mean (SEM). The center line in the box plots indicates the median, and the box edges represent the first and third quartiles. Adapted from Figure 2 of Paper II.

Based on genetic diversity, our isolates were classified into 28 MLSTs (Fig. 7A), several of which were disseminated across the Fyrisån River over space and time. For example, ST69 was identified during March, July, and November in isolates from all locations except Lake Mälaren. Additionally, ST88 and ST1193 were detected near the effluents of the WWTP and dWWTP in May and November, while ST131 was exclusively found at the dWWTP in March and November (Fig. 7B-C). These findings suggest that the WWTP is potentially a key contributor to the spread of STs across the river. Moreover, several globally distributed STs were identified. Notably, we detected strains belonging to well-documented STs associated with clinically relevant settings and multidrug resistance (MDR), such as ST38, ST131, and ST1193 [160-162]. Some STs are primarily associated with livestock, such as ST88158, and some have been reported in both human and animal sources, such as ST10, and ST69[160,163] suggesting potential zoonotic transmission. These results indicate possible dissemination from both anthropogenic and animal sources into Fyrisån River.

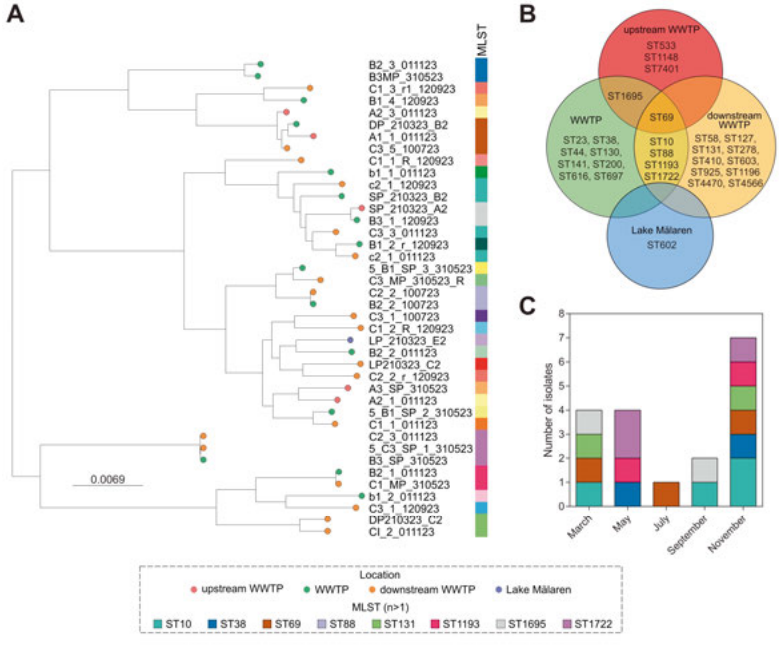

Figure 7. Genetic diversity and distribution of the 3GCR-Ec isolates across sampling sites and time points. (A) Maximum-likelihood phylogenetic tree reconstructed using RAxML based on 3,359 core genes shared by at least 95% of isolates. Node colors indicate sampling locations (red = upWWTP, green = WWTP, orange = dWWTP, blue = Mälaren). Leaf labels include metadata: sampling site (A–D), biological replicate (e.g., A1, A2), and sampling date (DDMMYY). **(B)** Venn diagram showing the overlap of multilocus sequence types (STs) among sites. **(C)** Bar plot displaying the temporal distribution of STs across sampling months. Adapted from Figure 3 of Paper II.

The in-silico analysis of ARGs for 3GCR-Ec isolates showed that the highest number of ARGs per isolate was 13 ARGs in July at WWTP and the lowest number was 3 ARGs in March at Lake Mälaren (Fig. 8A).

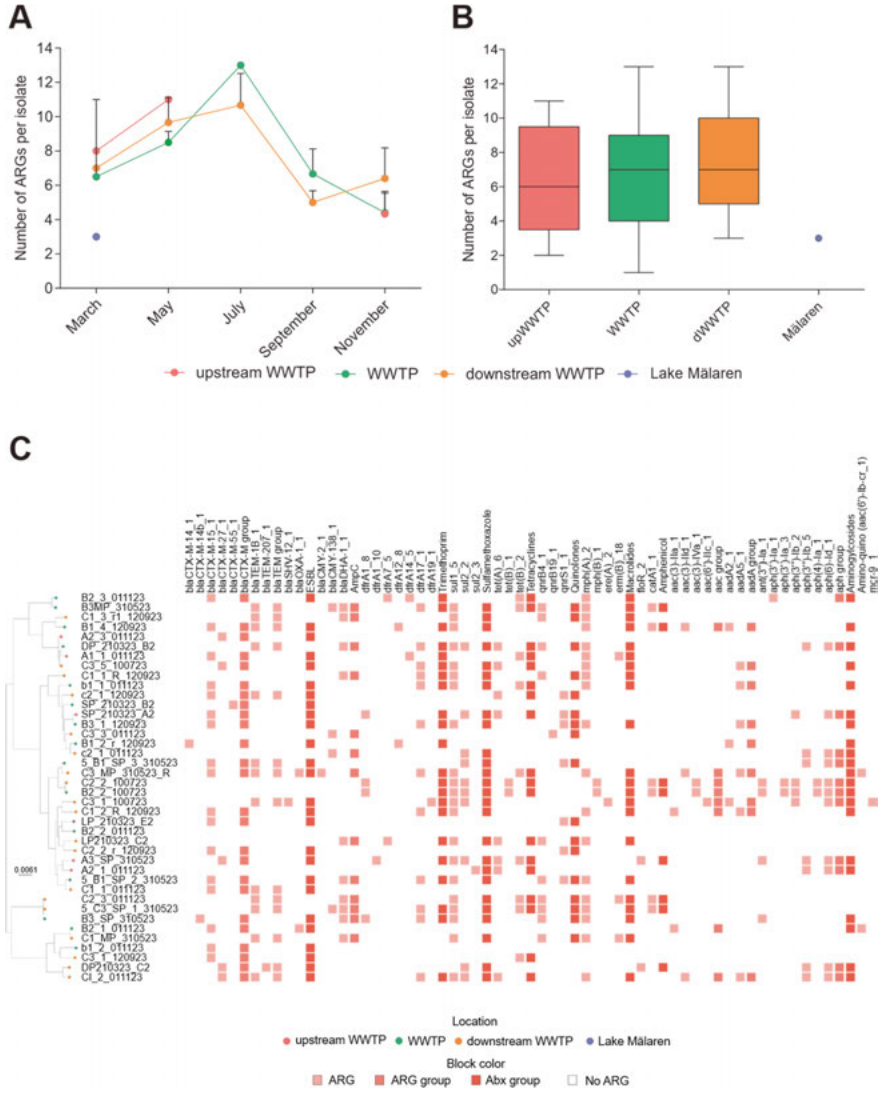

Figure 8. Distribution of ARGs among 3GCR-Ec isolates. (A) Temporal variation in the number of ARGs detected in each isolate per month across sampling points. Error bars represent the SEM. (B) Box plot showing ARG counts per isolate by location. (C) ARGs identified by ResFinder, along with the antibiotic classes and the genes they confer resistance to, are shown in different shades of red. The multidrug efflux pump gene mdf(A), present in all isolates, is not shown. Interactive visualization is available on Microreact: https://microreact.org/project/skYFEqgScJvssPkSrZJysc-fyrisan-project. Adapted from Figure 4 of Paper II,

This suggests that the potential risk of ARGs distribution in the Lake is lower than in the river. Additionally, no statistically significant differences in the number of ARGs per isolate were found between locations (Fig. 8B). This lack of significance may be attributed to the low volume of isolates, which will be considered in future studies. The in-silico analysis also showed that 65% were ESBL-Ec strains, with $bla_{CTX\text{-}M\text{-}15}$ being the most common ESBL gene (43%) (Fig. 8C, Table S3 of Paper II). Previous studies indicated that $bla_{CTX\text{-}M\text{-}15}$ is commonly associated with clinically relevant strains[72,164,165], and in some cases has been detected in environmental water[164]. This suggests that the gene in our study may originate from anthropogenic sources, such as hospital effluents or asymptomatic carriage in humans and animals, and is likely discharged into the river via WWTPs. These findings highlight the role of WWTPs as contaminating sources of antibiotic-resistant enteric bacteria, even in HICs with relatively well-developed sewage treatment and strict regulation of antibiotic use, consistent with the findings of Fagerströmet et al.[166]. This underscores the need to strengthen combined efforts to enhance surveillance, mitigate bacterial resistance in environmental water, and improve WWTPs.

Paper III. Genomic insights into ESBL- and pAmpC-producing *Escherichia coli* transmission between humans and livestock in rural Cambodia.

This study examined the genetic determinants for resistance and HGT, as well as the clonal relationships in ESC-Ec strains from asymptomatic carriage in humans and livestock in rural Cambodia. Additionally, the study underscores the importance of the One Health approach, which recognizes the interconnectedness of human, animal, and environmental health in addressing AMR. Fecal samples were collected from humans and animals in 100 households across different villages for microbiological analysis. 108 ESC-Ec isolates were subjected to WGS and in-silico analysis (Table S1).

Based on Clermont typing, the isolates (n=108) clustered into six known *E. coli* phylogroups (A, B1, B2, C, D, F) (Fig. 9B). Most of our isolates from humans and animals (78.7%) belonged to phylogroups A and B1, which are commonly occurring as commensal strains in both humans and livestock[167]. Also, phylogroup B2 was only found in human isolates, similar to previous

studies[167,168]. The genomes were classified into 50 STs based on MLST identification, with three genomes assigned a novel ST (ST15565) (Paper III, Table S1). Seventeen STs were found in both humans and animals, with ST155 being the most prevalent (11/108; 10.2%), isolated from poultry (n=5), humans (n=5), and a ruminant (n=1) (Paper III Table S1).

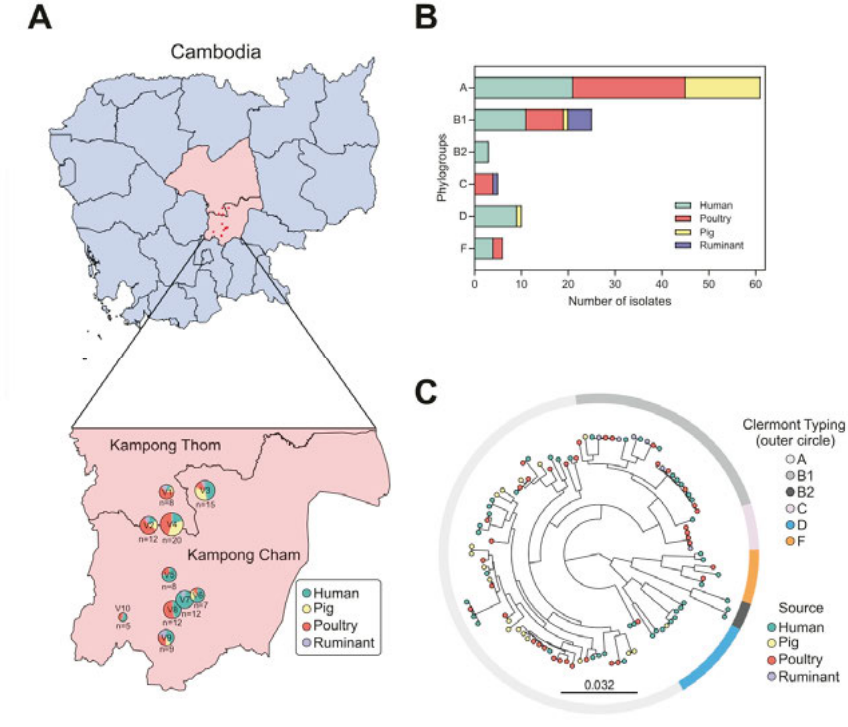

Figure 9. **Geographic and phylogenetic distribution of ESC-Ec isolates (n=108) from humans and livestock in rural Cambodia**. (A) Map of village sampling locations (V1–V10) highlighted in red. The zoom-in shows the distribution of ESC-Ec strains from humans and livestock. (B) Distribution of isolates across six known *E. coli* phylogroups by host species. (C) Maximum-likelihood phylogeny based on 282,923 core SNPs, with leaf colors indicating host origin and outer ring showing phylogroup classification. Adapted from Figure 1 of Paper III

This finding indicates that some strains are circulating between humans and livestock across households and villages in rural Cambodia (Paper III, Supplementary Fig. 1A, Table S1).

For greater resolution of genomic associations than MLST, we used cgMLST to identify the genetic similarity between isolates from different sources and villages. In our study, 21 out of 108 isolates were similar and clustered into ten cgMLST found across households and villages. For instance, isolates belonging to ST271555 were obtained from ruminants and poultry of different households in the same village. Additionally, isolates belonging to ST271559 were obtained from humans and poultry of different villages. Furthermore, isolates belonging to ST271624 were found in humans across different villages (Fig. 10A-C). These findings indicate clonal transmission, i.e., certain strains are spreading between different hosts and locations.

Our results are consistent with the findings of inter-household and inter-host transmission patterns reported in Kenya[118].

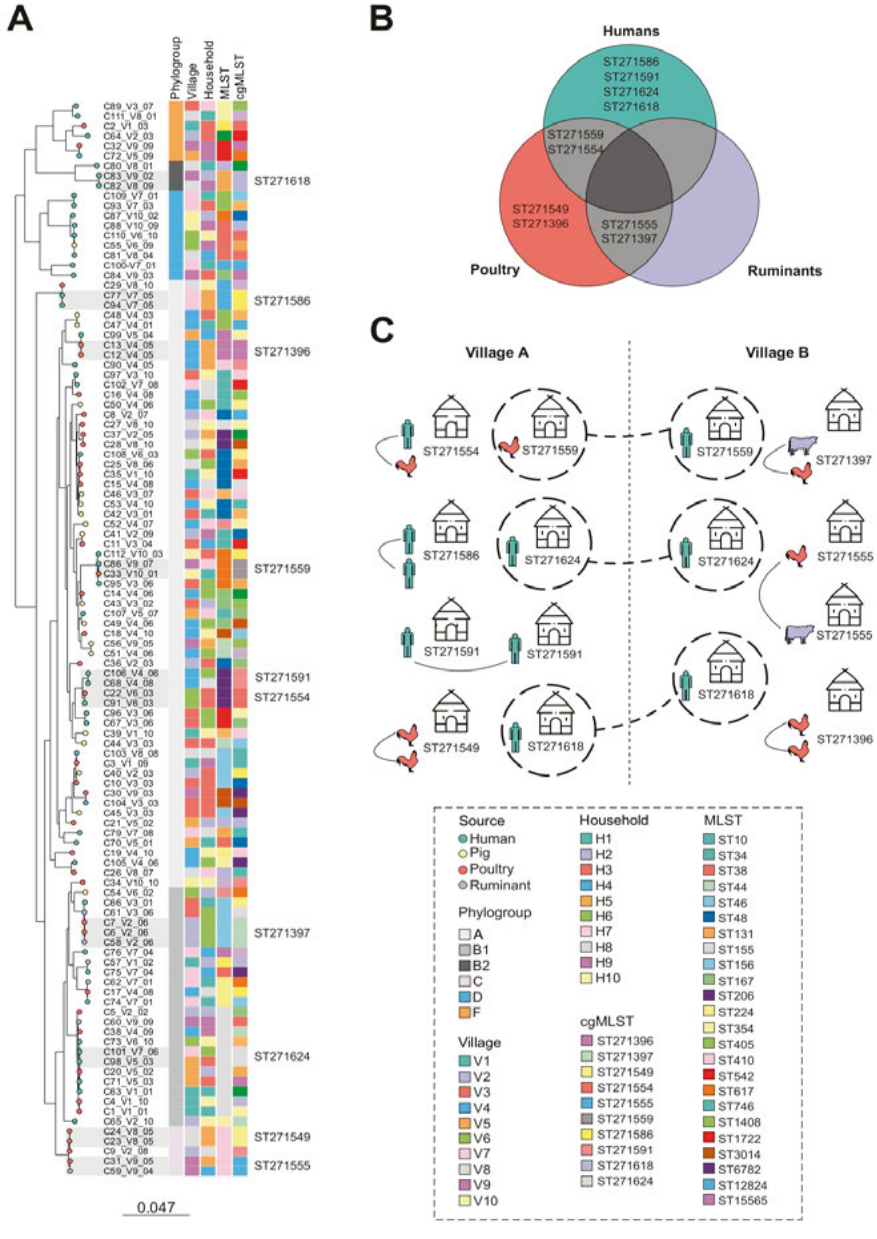

Figure 10. Clonal relationships and transmission patterns of ESC-Ec isolates across hosts and villages. (A) A maximum-likelihood phylogenetic tree was reconstructed for 108 ESC-Ec isolates using RAxML based on 282923 core SNPs shared by at least 95% of isolates. The tree was annotated with sample ID, village, and household metadata. Ten highly similar cgSTs shared across hosts and locations are shaded in grey. And the STs based on MLST, cgMLST, phylogroup, and household metadata are indicated as colored blocks next to the phylogenetic tree. (B) Venn diagram showing cgSTs shared between humans and livestock. (C) Schematic illustrating the distribution of shared cgSTs across households and villages. Adapted from Figure 2. of Paper III.

The genomes were also screened for the presence of AMR genes (Fig. 11) using ResFinder[140,141]. Nearly all ESC-Ec isolates, 98.1% (106/108) (Paper III, Table S4), were MDR, consistent with the findings of Chirindze et al.[169]. ESBL genes, specifically *bla*$_{CTX-M}$, were found in 84.2% (91/108) of isolates, predominantly those isolated from humans (93.5%) (Table 3).

Table 3. Distribution of most common ARGs in ESC-Ec isolates from humans and animals**.** This table is adapted from Table 1 of Paper III

Antibiotic class	**Major AMR gene group**	**Number of isolates (%)**				**Total gene number**
		Humans (*n*=46)	**Poultry (*n*=38)**	**Pigs (*n*=18)**	**Ruminants (*n*=6)**	
beta-lactams	bla$_{CTX-M}$	43 (93.5%)	29 (76.3%)	15 (83.3%)	4 (66.7%)	91
	bla$_{TEM}$	34 (73.9%)	28 (73.9%)	12 (66.7%)	5 (83.3%)	79
Tetracyclines	*tet(A)*	32 (69.6%)	25 (65.8%)	12 (66.7%)	3 (50.0%)	72
Sulfonamides	*sul2*	32 (69.6%)	22 (57.9%)	14 (77.8%)	2 (33.3%)	70
Trimethoprim	*dfrA14*	20 (43.5%)	19 (50%)	7 (38.9%)	2 (33.3%)	48
Aminoglycosides	*aph(3'')-Ib*	27 (58.7%)	18 (47.4%)	12 (66.7%)	2 (33.3%)	59
	aadA2	15 (32.6%)	13 (34.2%)	7 (38.9%)	1 (16.7%)	36
	aac(3)-IId	8 (17.4%)	6 (15.8%)	4 (22.2%)	1 (16.7%)	19
	ant(2'')-Ia	1 (2.2%)	2 (5.3%)	0	0	3
Amphenicol	*catA*	20 (43.5%)	14 (36.9%)	11 (61.1%)	3 (50%)	48
	cmlA1	7 (15.2%)	6 (15.8%)	5 (27.8%)	1 (16.7%)	19
	floR	8 (17.4%)	0	3 (16.7%)	0	11
Fluoroquinolones	*qnrS1*	17 (37%)	19 (50%)	8 (44.4%)	1 (16.7%)	45
	qepA1	2 (4.3%)	1 (2.6%)	0	0	3
Lincosamides	*lnu(F)*	10 (21.7%)	9 (23.7%)	4 (22.2%)	0	23
Macrolides	*mph(A)*	24 (52.2%)	12 (31.6%)	8 (44.4%)	2 (33.3%)	46
Colistin	*mcr-1.1*	1 (2.2%)	3 (7.9%)	3 (16.7%)	0	7
Aminoglycoside-Quinolones	*aac(6')-Ib-cr*	4 (8.7%)	3 (7.9%)	1 (5.5%)	1 (16.7%)	9

Among the environmental isolates, the most common CTX-M subtype was CTX-M-55 (29.6%) (Fig. 11, Paper III. Table S2), which was previously reported in clinical isolates in Thailand[170]. Our findings also correspond to a previous study from Vietnam[171], indicating the widespread presence of this gene in the environment. Resistance to tetracycline, trimethoprim, and sulfonamide was also observed among these isolates. The resistance genes *tet, dfr*, and *sul* were found in over 85% of all isolates, aligning with global research findings[172-175].

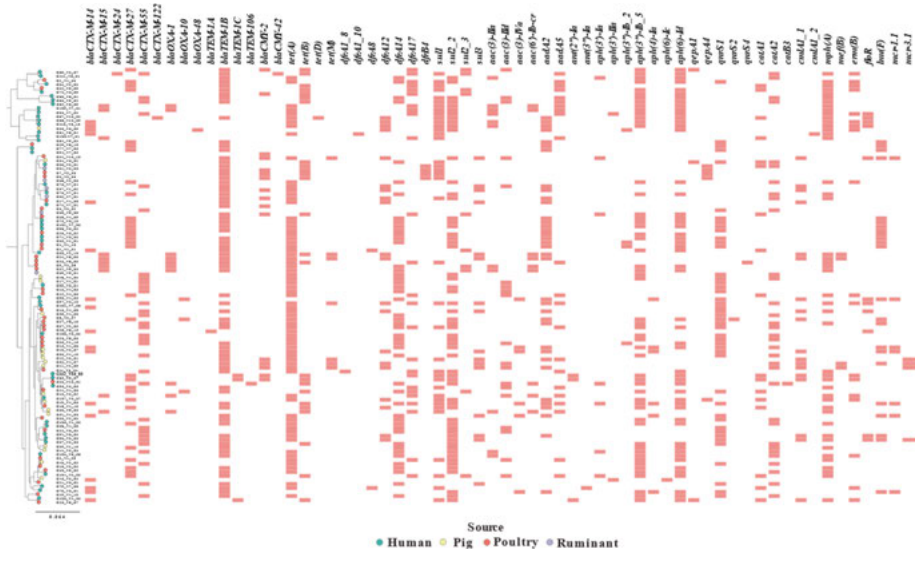

Figure 11. Presence of ARGs in ESC-Ec isolates from human and livestock sources. The same phylogenetic tree is colored by host species (green = human, yellow = pig, red = poultry, violet = ruminant). Red blocks indicate the presence of ARGs identified using ResFinder. The scale bar represents the number of substitutions per site. Adopted from Figure 3 of Paper III

The high prevalence of IncF plasmids among our ESC-Ec isolates (75.9%, 82/108; Table 4) underscores their potential role in HGT, contributing to the dissemination of ARGs. This observation aligns with previous studies identifying IncF plasmids as key vectors in ARG transmission[88,103].

Table 4. Plasmid distribution in ESC-Ec from humans and livestock.This table is adapted from Table S5 of Paper III.

Plasmid families	Humans (n=46)	Poultry (n=38)	Pigs (n=18)	Ruminants (n=6)	Total plasmid occurrence
IncF (family)	35 (76.1%)	26 (68.4%)	17 (94.4%)	4 (66.7%)	82
Col (family)	17 (37%)	14 (36.8%)	6 (33.3%)	2 (33.3%)	39
p0111	10 (21.7%)	13 (34.2%)	3 (16.7%)	1 (16.7%)	27
IncX (family)	7 (15.2%)	9 (23.7%)	6 (33.3%)	1 (16.7%)	23
IncH (family)	4 (8.7%)	12 (31.6%)	4 (22.2%)	0	20
IncQ1	6 (13%)	3 (7.9%)	2 (11.1%)	1 (16.7%)	12
IncR	4 (8.7%)	3 (7.9%)	2 (11.1%)	1 (16.7%)	10
Incl (family)	4 (8.7%)	2 (5.3%)	0	0	6
IncY	3 (6.5%)	1 (2.6%)	0	1 (16.7%)	5
IncN	0	1 (2.6%)	0	1 (16.7%)	2
IncB/O/K/Z	1 (2.2%)	0	0	0	1

The most prevalent co-occurrence of AMR genes was observed for the *aph(3'')-Ib–sul2* found in 50% of ESC-Ec isolates, highlighting the high potential for horizontal gene transfer (HGT) among the sources (Paper III, Table S6). These findings highlight the high risk of AMR transmission, emphasizing the urgent need to mitigate its spread among different sources.

To the best of our knowledge, this is the first published report of an in-silico analysis in Cambodia to study the genetic relationships and resistance profiles of ESC-Ec isolated from different sources. This method enables a comprehensive understanding of the patterns of ARG transmission and the identification of clonal relationships. However, the sample size and geographic scope of our study may limit the generalizability of the findings.

Conclusions and future perspectives

Enteric bacteria, such as *E. faecium*, *E. faecalis*, and *E. coli*, are commonly used as fecal indicators for assessing water quality. They can also act as important pathogens, playing significant roles in the spread of AMR from a One Health perspective. Our findings underscore the interconnectedness between humans, livestock, and the environment in the spread of AMR. In this thesis, we reported that the dissemination of FIB (*E. faecalis*, *E. faecium*, and *E. coli*) was higher near the WWTP outlet in Örebro. While in Uppsala, we focused on the abundance of 3GCR-Ec and found higher concentrations of these resistant strains just downstream of WWTP. Our findings indicate that the WWTP is potentially a significant source of FIB and 3GCR-Ec distribution in Swedish urban rivers. MDR was observed in *E. coli*, *E. faecium*, and *E. faecalis*, with *E. coli* producing extended-spectrum beta-lactamases. Additionally, *E. faecium* exhibited vancomycin resistance, although it was not classified as MDR. These findings from relatively clean urban areas suggest that fecal contamination and AMR likely originate within the community and are subsequently dispersed via WWTP effluent. The presence of MDR and ESBL-Ec strains, harboring common ESBL genes (*bla*$_{CTX\text{-}M\text{-}15}$ and *bla*$_{CTX\text{-}M\text{-}55}$), suggests contamination from anthropogenic sources. This contamination can contribute to rising ESBL carriage and infections within the population, highlighting the global spread of antibiotic resistance. Therefore, enhanced surveillance of ESBL-Ec in WWTP effluents and receiving water bodies, along with improved wastewater treatment technologies, is recommended, even in areas considered relatively unpolluted. Monitoring antibiotic resistance in FIB is essential for evaluating the global state of antibiotic resistance.

In rural Cambodia, human and animal reservoirs for transmitting ESC-Ec and associated AMR genes were investigated. Evidence for clonal spread and HGT between these reservoirs was found. The widespread prevalence of MDR strains across households and villages highlights the urgent need for antimicrobial stewardship programs and infection control measures using a One Health approach, especially in LMICs.

Overall, this thesis demonstrates the importance of a One Health approach in understanding and mitigating the spread of antibiotic resistance. By

improving and monitoring WWTP quality, targeting humans and livestock, we can develop effective strategies to limit the spread of resistant pathogens and reduce the public health burden of AMR.

Regarding future perspectives, we are considering evaluating the antibiotic resistance in *E. coli* and enterococci isolated from birds near the bird sanctuary in Uppsala, as these birds are known to be significant sources of fecal contamination in surface water. This could be done by PCR-based screening of *E. avium*, *E. gallinarum,* and *E. coli* from freshly fecal bird samples*,* followed by in vitro and in-silico antibiotic resistance analysis. Another future goal is to determine the bacterial diversity and characterize the resistome among bacterial communities in Swedish waters using metagenomic analysis. Metagenomics is a powerful tool that has the potential to provide a deeper understanding of the temporal dynamics in microbial communities, which is essential for environmental management and public health. To this end, we have collected water samples from the Fyrisån river and Svandammen pond in Uppsala and sent gDNA samples for Shotgun Metagenomic Sequencing. The two studies aim to analyze microbial diversity and further characterize antibiotic resistance in Swedish urban waters.

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References:

1. Centers for Disease Control and Prevention. One Health. (2023).
2. Wilcox, B.A. & Steele, J.A. One health and emerging zoonotic diseases: Framework, integration and challenges. *Handbook of global health*, 1-49 (2020).
3. Mittal, A.K., Bhardwaj, R., Mishra, P. & Rajput, S.K. Antimicrobials misuse/overuse: adverse effect, mechanism, challenges and strategies to combat resistance. *The Open Biotechnology Journal* **14**(2020).
4. Van de Vuurst, P. & Escobar, L.E. Climate change and infectious disease: a review of evidence and research trends. *Infectious Diseases of Poverty* **12**, 51 (2023).
5. Garcia, S.N., Osburn, B.I. & Jay-Russell, M.T. One health for food safety, food security, and sustainable food production. *Frontiers in Sustainable Food Systems* **4**, 1 (2020).
6. WHO, FAO, WOAH & UNEP. *One Health Joint Plan of Action (2022–2026): Working together for the health of humans, animals, plants and the environment*, (World Health Organization, 2022).
7. United Nations Environment Programme. UNEP One Health. Vol. 2025 (2022).
8. Yopa, D.S.*, et al.* Barriers and enablers to the implementation of one health strategies in developing countries: a systematic review. *Frontiers in public health* **11**, 1252428 (2023).
9. Abbas, S.S., Shorten, T. & Rushton, J. Meanings and mechanisms of One Health partnerships: insights from a critical review of literature on cross-government collaborations. *Health Policy and Planning* **37**, 385-399 (2022).
10. Silva, N., Igrejas, G., Gonçalves, A. & Poeta, P. Commensal gut bacteria: distribution of Enterococcus species and prevalence of Escherichia coli phylogenetic groups in animals and humans in Portugal. *Annals of microbiology* **62**, 449-459 (2012).
11. Elsner, H.-A.*, et al.* Virulence Factors of Enterococcus faecalis and Enterococcus faecium Blood Culture Isolates. *European Journal of Clinical Microbiology & Infectious Diseases* **19**, 39-42 (2000).
12. Jett, B.D., Huycke, M.M. & Gilmore, M.S. Virulence of enterococci. *Clinical microbiology reviews* **7**, 462-478 (1994).

13. Russo, T.A. & Johnson, J.R. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. *Microbes and infection* **5**, 449-456 (2003).
14. Arias, C.A. & Murray, B.E. The rise of the Enterococcus: beyond vancomycin resistance. *Nature Reviews Microbiology* **10**, 266-278 (2012).
15. Johnson, A.O*., et al.* Function and contribution of two putative Enterococcus faecalis glycosaminoglycan degrading enzymes to bacteremia and catheter-associated urinary tract infection. *Infection and Immunity*, e00199-00124 (2024).
16. Rahimi, F., Talebi, M., M, S. & Pourshafie, M.R. Distribution of enterococcal species and detection of vancomycin resistance genes by multiplex PCR in Tehran sewage. *Iranian biomedical journal* **11**, 161–167 (2007).
17. Grammenou, P., Spiliopoulou, I., Sazakli, E. & Papapetropoulou, M. PFGE analysis of enterococci isolates from recreational and drinking water in Greece. *Journal of Water and Health* **4**, 263-269 (2006).
18. Rathnayake, I., Hargreaves, M. & Huygens, F. SNP diversity of Enterococcus faecalis and Enterococcus faecium in a South East Queensland waterway, Australia, and associated antibiotic resistance gene profiles. *BMC Microbiology* **11**, 201 (2011).
19. Kinzelman, J., Ng, C., Jackson, E., Gradus, S. & Bagley, R. Enterococci as Indicators of Lake Michigan Recreational Water Quality: Comparison of Two Methodologies and Their Impacts on Public Health Regulatory Events. *Applied and Environmental Microbiology* **69**, 92-96 (2003).
20. European Environment Agency. European bathing water quality in 2011. (Denmark, 2012).
21. Murray, C.J*., et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet* **399**, 629-655 (2022).
22. Camilleri, S., Tsai, D., Langham, F., Ullah, S. & Chiong, F. Epidemiology, clinical outcomes and risk factors of third-generation cephalosporin-resistant Escherichia coli hospitalized infections in remote Australia—a case–control study. *JAC-Antimicrobial Resistance* **5**, dlad138 (2023).
23. Hayer, S.S*., et al.* Global distribution of extended spectrum cephalosporin and carbapenem resistance and associated resistance markers in Escherichia coli of swine origin–a systematic review and meta-analysis. *Frontiers in microbiology* **13**, 853810 (2022).
24. Seiffert, S.N., Hilty, M., Perreten, V. & Endimiani, A. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? *Drug Resistance Updates* **16**, 22-45 (2013).

25. Brouwer, A.F., Masters, N.B. & Eisenberg, J.N. Quantitative microbial risk assessment and infectious disease transmission modeling of waterborne enteric pathogens. *Current environmental health reports* **5**, 293-304 (2018).
26. Tsai, K.*, et al.* Enteric pathogen diversity in infant foods in low-income neighborhoods of Kisumu, Kenya. *International journal of environmental research and public health* **16**, 506 (2019).
27. Hoffmann, S.*, et al.* Attribution of global foodborne disease to specific foods: Findings from a World Health Organization structured expert elicitation. *PloS one* **12**, e0183641 (2017).
28. Omulo, S., Mugoh, M., Obiya, J., Alando, M. & Call, D.R. Estimating the population-level prevalence of antimicrobial-resistant enteric bacteria from latrine samples. *Antimicrobial Resistance & Infection Control* **11**, 106 (2022).
29. Tyagi, I., Tyagi, K., Ahamad, F., Bhutiani, R. & Kumar, V. Assessment of Bacterial Community Structure, Associated Functional Role, and Water Health in Full-Scale Municipal Wastewater Treatment Plants. *Toxics* **13**, 3 (2024).
30. Bonetta, S.*, et al.* Impact of wastewater treatment plants on microbiological contamination for evaluating the risks of wastewater reuse. *Environmental Sciences Europe* **34**, 20 (2022).
31. Federigi, I.*, et al.* Wastewater Treatment Plants Performance for Reuse: Evaluation of Bacterial and Viral Risks. *Water* **16**, 1399 (2024).
32. Li, Q.*, et al.* Tracking spatio-temporal distribution and transmission of antibiotic resistance in aquatic environments by using ESBL-producing Escherichia coli as an indicator. *Journal of Environmental Management* **344**, 118534 (2023).
33. Azuma, T., Uchiyama, T., Zhang, D., Usui, M. & Hayashi, T. Distribution and characteristics of carbapenem-resistant and extended-spectrum β-lactamase (ESBL) producing Escherichia coli in hospital effluents, sewage treatment plants, and river water in an urban area of Japan. *Science of The Total Environment* **839**, 156232 (2022).
34. Cheng, H.-W.A.*, et al.* Municipal wastewater treatment plants as pathogen removal systems and as a contamination source of noroviruses and Enterococcus faecalis. *Journal of water and health* **10**, 380-389 (2012).
35. Bendz, D., Paxéus, N.A., Ginn, T.R. & Loge, F.J. Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden. *Journal of Hazardous Materials* **122**, 195-204 (2005).
36. Khalaf, H.*, et al.* In vitro analysis of inflammatory responses following environmental exposure to pharmaceuticals and inland waters. *Science of The Total Environment* **407**, 1452-1460 (2009).

37. Li, W. & Wang, A. Genomic islands mediate environmental adaptation and the spread of antibiotic resistance in multiresistant Enterococci-evidence from genomic sequences. *BMC microbiology* **21**, 1-10 (2021).
38. Iversen, A., Kühn, I., Franklin, A. & Möllby, R. High Prevalence of Vancomycin-Resistant Enterococci in Swedish Sewage. *Applied and Environmental Microbiology* **68**, 2838-2842 (2002).
39. Ghazvinian, M.*, et al.* Antimicrobial resistance patterns, virulence genes, and biofilm formation in enterococci strains collected from different sources. *BMC Infectious Diseases* **24**, 274 (2024).
40. Biswas, S.*, et al.* Antibiotic resistance in toxigenic E. coli: a severe threat to global health. *Discover Medicine* **1**, 72 (2024).
41. Rahimi, F., Talebi, M., SEYFI, M. & POURSHAFIEI, M. Distribution of enterococcal species and detection of vancomycin resistance genes by multiplex PCR in Tehran sewage. (2007).
42. Wade, T.J.*, et al.* Rapidly Measured Indicators of Recreational Water Quality Are Predictive of Swimming-Associated Gastrointestinal Illness. *Environmental Health Perspectives* **114**, 24-28 (2006).
43. Joosten, R.*, et al.* Risk factors for gastroenteritis associated with canal swimming in two cities in the Netherlands during the summer of 2015: A prospective study. *PLoS One* **12**, e0174732 (2017).
44. Madigan, M.T.a.M., John M. *Brock Biology of Microorganisms*, (Pearson Education, Inc., United States of America, 2006).
45. Wald-Dickler, N., Holtom, P. & Spellberg, B. Busting the myth of "static vs cidal": a systemic literature review. *Clinical infectious diseases* **66**, 1470-1474 (2018).
46. Ishak, A., Mazonakis, N., Spernovasilis, N., Akinosoglou, K. & Tsioutis, C. Bactericidal versus bacteriostatic antibacterials: clinical significance, differences and synergistic potential in clinical practice. *Journal of Antimicrobial Chemotherapy* **80**, 1-17 (2025).
47. Bush, K. & Bradford, P.A. β-Lactams and β-lactamase inhibitors: an overview. *Cold Spring Harbor perspectives in medicine* **6**, a025247 (2016).
48. Finberg, R.W.*, et al.* The importance of bactericidal drugs: future directions in infectious disease. *Clinical infectious diseases* **39**, 1314-1320 (2004).
49. Finberg, R.W.*, et al.* The Importance of Bactericidal Drugs: Future Directions in Infectious Disease. *Clinical Infectious Diseases* **39**, 1314-1320 (2004).
50. Etebu, E. & Arikekpar, I. Antibiotics: Classification and mechanisms of action with emphasis on molecular perspectives. *International journal of applied microbiology and biotechnology research* **4**, 90-101 (2016).

51. Manyi-Loh, C., Mamphweli, S., Meyer, E. & Okoh, A. Antibiotic use in agriculture and its consequential resistance in environmental sources: potential public health implications. *Molecules* **23**, 795 (2018).
52. Li, D.*, et al.* Antibiotic Resistance Characteristics of Environmental Bacteria from an Oxytetracycline Production Wastewater Treatment Plant and the Receiving River. *Applied and Environmental Microbiology* **76**, 3444-3451 (2010).
53. Jacoby, G.A. & Munoz-Price, L.S. The New β-Lactamases. *New England Journal of Medicine* **352**, 380-391 (2005).
54. Paterson, D.L. & Bonomo, R.A. Extended-Spectrum β-Lactamases: a Clinical Update. *Clinical Microbiology Reviews* **18**, 657-686 (2005).
55. Giedraitienė, A., Vitkauskienė, A., Naginienė, R. & Pavilonis, A. Antibiotic Resistance Mechanisms of Clinically Important Bacteria. *Medicina* **47**, 19 (2011).
56. Alekshun, M.N. & Levy, S.B. Molecular Mechanisms of Antibacterial Multidrug Resistance. *Cell* **128**, 1037-1050 (2007).
57. Džidić, S., Šušković, J. & Kos, B. Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects. *Food Technology & Biotechnology* **46**(2008).
58. Jonas, B.M., Murray, B.E. & Weinstock, G.M. Characterization of *emeA* , a *norA* Homolog and Multidrug Resistance Efflux Pump, in *Enterococcus faecalis*. *Antimicrobial Agents and Chemotherapy* **45**, 3574-3579 (2001).
59. Costache, C.*, et al.* CRISPR-Cas System, Antimicrobial Resistance, and Enterococcus Genus—A Complicated Relationship. *Biomedicines* **12**, 1625 (2024).
60. Tenover, F.C. Mechanisms of antimicrobial resistance in bacteria. *American Journal of Infection Control* **34**, S3-S10 (2006).
61. Hall, B.G. & Barlow, M. Revised Ambler classification of β-lactamases. *Journal of Antimicrobial Chemotherapy* **55**, 1050-1051 (2005).
62. Bush, K., Jacoby, G.A. & Medeiros, A.A. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial agents and chemotherapy* **39**, 1211-1233 (1995).
63. Dadashpour, R., Moghaddam, M.J.M. & Salehi, Z. Prevalence of non-extended spectrum β-lactamases SHV-1 and TEM-1 or-2 types in multidrug-resistant Enterobacteriaceae in northern Iran. *Biologia Futura* **71**, 419-426 (2020).
64. Abay, G.K.*, et al.* Extended-spectrum β-lactamase production and antimicrobial resistance among Enterobacteriaceae causing clinical infections in Africa: a systematic review and meta-analysis (2012–2020). *European Journal of Medical Research* **30**, 1-14 (2025).

65. Kliebe, C., Nies, B., Meyer, J., Tolxdorff-Neutzling, R. & Wiedemann, B. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. *Antimicrobial agents and chemotherapy* **28**, 302-307 (1985).
66. Kaye, K.S. & Belley, A. Third-generation cephalosporin-resistant Enterobacterales are critical priority pathogens, too! *Antimicrobial agents and chemotherapy* **66**, e00213-00222 (2022).
67. Kettani Halabi, M.*, et al.* Antibiotic resistance pattern of extended spectrum beta lactamase producing Escherichia coli isolated from patients with urinary tract infection in Morocco. *Frontiers in Cellular and Infection Microbiology* **11**, 720701 (2021).
68. Woerther, P.-L., Burdet, C., Chachaty, E. & Andremont, A. Trends in human fecal carriage of extended-spectrum β-lactamases in the community: toward the globalization of CTX-M. *Clinical microbiology reviews* **26**, 744-758 (2013).
69. Cassini, A.*, et al.* Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. *The Lancet infectious diseases* **19**, 56-66 (2019).
70. Phungoen, P.*, et al.* The association of ESBL Escherichia coli with mortality in patients with Escherichia coli bacteremia at the emergency department. *Drug Target Insights* **16**, 12 (2022).
71. Shurina, B.A. & Page, R.C. Structural comparisons of cefotaximase (CTX-M-ase) sub family 1. *Frontiers in Microbiology* **12**, 688509 (2021).
72. Bevan, E.R., Jones, A.M. & Hawkey, P.M. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *Journal of antimicrobial chemotherapy* **72**, 2145-2155 (2017).
73. Yang, J.-T., Zhang, L.-J., Lu, Y., Zhang, R.-M. & Jiang, H.-X. Genomic insights into global bla CTX-M-55-positive Escherichia coli epidemiology and transmission characteristics. *Microbiology spectrum* **11**, e01089-01023 (2023).
74. Higgins, O.*, et al.* Portable differential detection of CTX-M ESBL gene variants, bla CTX-M-1 and bla CTX-M-15, from Escherichia coli isolates and animal fecal samples using loop-primer endonuclease cleavage loop-mediated isothermal amplification. *Microbiology spectrum* **11**, e03316-03322 (2023).
75. Atterby, C.*, et al.* ESBL-producing Escherichia coli in Swedish gulls—a case of environmental pollution from humans? *PloS one* **12**, e0190380 (2017).
76. Sidjabat, H.E.*, et al.* Molecular epidemiology of CTX-M-producing Escherichia coli isolates at a tertiary medical center in western Pennsylvania. *Antimicrobial agents and chemotherapy* **53**, 4733-4739 (2009).

77. Abdelrahim, S.S., Fouad, M., Abdallah, N., Ahmed, R.F. & Zaki, S. Comparative study of CTX-M-15 producing Escherichia coli ST131 clone isolated from urinary tract infections and acute diarrhoea. *Infection and Drug Resistance*, 4027-4038 (2021).
78. Al-Agamy, M.H.*, et al.* Molecular characteristics of extended-spectrum β-lactamase-producing Escherichia coli in Riyadh: emergence of CTX-M-15-producing E. coli ST131. *Annals of clinical microbiology and antimicrobials* **13**, 1-7 (2014).
79. Yang, J., Zhang, L., Lu, Y., Zhang, R. & Jiang, H. Genomic insights into global blaCTX-M-55-positive Escherichia coli epidemiology and transmission characteristics, Microbiol. Spectr. 11 (4)(2023) e01089–e10123.
80. Zeng, S.*, et al.* Molecular epidemiology and characteristics of CTX-M-55 extended-spectrum β-lactamase-producing Escherichia coli from Guangzhou, China. *Frontiers in Microbiology* **12**, 730012 (2021).
81. Micenková, L.*, et al.* Characterization of human uropathogenic ESBL-producing Escherichia coli in the Czech Republic: spread of CTX-M-27-producing strains in a university hospital. *Microbial Drug Resistance* **20**, 610-617 (2014).
82. Doumith, M., Dhanji, H., Ellington, M.J., Hawkey, P. & Woodford, N. Characterization of plasmids encoding extended-spectrum β-lactamases and their addiction systems circulating among Escherichia coli clinical isolates in the UK. *Journal of antimicrobial chemotherapy* **67**, 878-885 (2012).
83. Wang, X.*, et al.* Inter-plasmid transfer of antibiotic resistance genes accelerates antibiotic resistance in bacterial pathogens. *The ISME journal* **18**, wrad032 (2024).
84. Peter, S.*, et al.* Tracking of antibiotic resistance transfer and rapid plasmid evolution in a hospital setting by Nanopore sequencing. *Msphere* **5**, 10.1128/msphere. 00525-00520 (2020).
85. Castañeda-Barba, S., Top, E.M. & Stalder, T. Plasmids, a molecular cornerstone of antimicrobial resistance in the One Health era. *Nature Reviews Microbiology* **22**, 18-32 (2024).
86. Coluzzi, C. & Rocha, E.P. The Spread of Antibiotic Resistance Is Driven by Plasmids Among the Fastest Evolving and of Broadest Host Range. *Molecular Biology and Evolution* **42**, msaf060 (2025).
87. Mahmud, B.*, et al.* Epidemiology of plasmid lineages mediating the spread of extended-spectrum beta-lactamases among clinical Escherichia coli. *Msystems* **7**, e00519-00522 (2022).
88. Sivaraman, G.K.*, et al.* Antibiotic resistance profiles and molecular characteristics of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae isolated from shrimp aquaculture farms in Kerala, India. *Frontiers in Microbiology* **12**, 622891 (2021).

89. Benz, F.*, et al.* Plasmid-and strain-specific factors drive variation in ESBL-plasmid spread in vitro and in vivo. *The ISME journal* **15**, 862-878 (2021).
90. Magiorakos, A.-P.*, et al.* Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection* **18**, 268-281 (2012).
91. Kim, M., Park, J., Kang, M., Yang, J. & Park, W. Gain and loss of antibiotic resistant genes in multidrug resistant bacteria: One Health perspective. *Journal of microbiology* **59**, 535-545 (2021).
92. Hawkey, P.M. The origins and molecular basis of antibiotic resistance. *BMJ* **317**, 657-660 (1998).
93. Poirel, L., Lagrutta, E., Taylor, P., Pham, J. & Nordmann, P. Emergence of metallo-β-lactamase NDM-1-producing multidrug-resistant Escherichia coli in Australia. *Antimicrobial agents and chemotherapy* **54**, 4914-4916 (2010).
94. Wipf, J. & Perreten, V. Discovery of novel MLSB resistance methylase genes and their associated genetic elements in staphylococci. Curr Clin Microbiol Rep 3: 42–52. (2016).
95. Founou, R.C., Founou, L.L., Allam, M., Ismail, A. & Essack, S.Y. Genome analysis of multidrug resistant Enterococcus faecium and Enterococcus faecalis circulating among hospitalized patients in uMgungundlovu District, KwaZulu-Natal, South Africa. *BMC Infectious Diseases* **24**, 671 (2024).
96. Yang, X.*, et al.* A conjugative IncI1 plasmid carrying erm (B) and bla CTX-M-104 that mediates resistance to azithromycin and cephalosporins. *Microbiology Spectrum* **9**, e00286-00221 (2021).
97. Tigen, E.T.*, et al.* Disease and Molecular Medicine. (2015).
98. Salah, F.D.*, et al.* Distribution of quinolone resistance gene (qnr) in ESBL-producing Escherichia coli and Klebsiella spp. in Lomé, Togo. *Antimicrobial Resistance & Infection Control* **8**, 1-8 (2019).
99. Liang, G.*, et al.* Co-occurrence of NDM-9 and MCR-1 in a human gut colonized Escherichia coli ST1011. *Infection and Drug Resistance*, 3011-3017 (2021).
100. Domingues, C.P., Rebelo, J.S., Dionisio, F. & Nogueira, T. Multi-drug resistance in bacterial genomes—A comprehensive bioinformatic analysis. *International Journal of Molecular Sciences* **24**, 11438 (2023).
101. Padilla, E.*, et al.* Klebsiella pneumoniae AcrAB efflux pump contributes to antimicrobial resistance and virulence. *Antimicrobial agents and chemotherapy* **54**, 177-183 (2010).
102. Webber, M.A. & Piddock, L.J. The importance of efflux pumps in bacterial antibiotic resistance. *Journal of antimicrobial chemotherapy* **51**, 9-11 (2003).

103. Rozwandowicz, M.*, et al.* Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy* **73**, 1121-1137 (2018).
104. Bezabih, Y.M.*, et al.* Comparison of the global prevalence and trend of human intestinal carriage of ESBL-producing Escherichia coli between healthcare and community settings: a systematic review and meta-analysis. *JAC-Antimicrobial Resistance* **4**, dlac048 (2022).
105. Tumbarello, M.*, et al.* Costs of bloodstream infections caused by Escherichia coli and influence of extended-spectrum-β-lactamase production and inadequate initial antibiotic therapy. *Antimicrobial agents and chemotherapy* **54**, 4085-4091 (2010).
106. Otaigbe, I.I. & Elikwu, C.J. Drivers of inappropriate antibiotic use in low-and middle-income countries. *JAC-antimicrobial resistance* **5**, dlad062 (2023).
107. Bayaba, S.*, et al.* High prevalence of multidrug resistant and extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections in the West region, Cameroon. *BMC Infectious Diseases* **25**, 115 (2025).
108. Sianipar, O., Asmara, W., Dwiprahasto, I. & Mulyono, B. Mortality risk of bloodstream infection caused by either Escherichia coli or Klebsiella pneumoniae producing extended-spectrum β-lactamase: a prospective cohort study. *BMC Research Notes* **12**, 1-7 (2019).
109. Sawa, T., Moriyama, K. & Kinoshita, M. Current status of bacteriophage therapy for severe bacterial infections. *Journal of Intensive Care* **12**, 44 (2024).
110. Pino-Angeles, A., Leveritt III, J.M. & Lazaridis, T. Pore structure and synergy in antimicrobial peptides of the magainin family. *PLoS computational biology* **12**, e1004570 (2016).
111. Carlos, C.*, et al.* Escherichia coli phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. *BMC microbiology* **10**, 1-10 (2010).
112. Patel, I.R.*, et al.* Draft genome sequences of the Escherichia coli reference (ECOR) collection. *Microbiology resource announcements* **7**, 10.1128/mra. 01133-01118 (2018).
113. Koh, X.P.*, et al.* Genetic and ecological diversity of Escherichia coli and cryptic Escherichia clades in subtropical aquatic environments. *Frontiers in Microbiology* **13**, 811755 (2022).
114. Nakamura, A.*, et al.* Molecular subtyping for source tracking of Escherichia coli using core genome multilocus sequence typing at a food manufacturing plant. *PLoS One* **16**, e0261352 (2021).
115. Liu, C.*, et al.* Escherichia coli ST131-H22 as a foodborne uropathogen. mBio 9. (2018).
116. Silva, A.*, et al.* Antimicrobial resistance and clonal lineages of Escherichia coli from food-producing animals. *Antibiotics* **12**, 1061 (2023).

117. Ludden, C.*, et al.* One Health genomic surveillance of Escherichia coli demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock. mBio 10: e02693-18. *Search Google Scholar Export Citation* (2019).
118. Muloi, D.M.*, et al.* Population genomics of Escherichia coli in livestock-keeping households across a rapidly developing urban landscape. *Nature microbiology* **7**, 581-589 (2022).
119. Rasko, D.A.*, et al.* The pangenome structure of Escherichia coli: comparative genomic analysis of E. coli commensal and pathogenic isolates. *Journal of bacteriology* **190**, 6881-6893 (2008).
120. Ludden, C.*, et al.* One health genomic surveillance of Escherichia coli demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock. *MBio* **10**, 10.1128/mbio. 02693-02618 (2019).
121. Rwego, I.B., Gillespie, T.R., Isabirye-Basuta, G. & Goldberg, T.L. High rates of Escherichia coli transmission between livestock and humans in rural Uganda. *Journal of Clinical Microbiology* **46**, 3187-3191 (2008).
122. Zingg, W.*, et al.* Implementation research for the prevention of antimicrobial resistance and healthcare-associated infections; 2017 Geneva infection prevention and control (IPC)-think tank (part 1). *Antimicrobial Resistance & Infection Control* **8**, 1-9 (2019).
123. Doernberg, S.B.*, et al.* Essential resources and strategies for antibiotic stewardship programs in the acute care setting. *Clinical Infectious Diseases* **67**, 1168-1174 (2018).
124. Vors, L., Raboisson, D. & Lhermie, G. Analysis of convergence between a unified One Health policy framework and imbalanced research portfolio. *Discover Public Health* **21**, 33 (2024).
125. Osbjer, K.*, et al.* Household practices related to disease transmission between animals and humans in rural Cambodia. *BMC Public Health* **15**, 476 (2015).
126. Atterby, C.*, et al.* Carriage of carbapenemase‐ and extended‐spectrum cephalosporinase‐producing Escherichia coli and Klebsiella pneumoniae in humans and livestock in rural Cambodia; gender and age differences and detection of blaOXA‐48 in humans. *Zoonoses and public health* **66**, 603-617 (2019).
127. Bollet, C., Gevaudan, M., De Lamballerie, X., Zandotti, C. & De Micco, P. A simple method for the isolation of chromosomal DNA from gram positive or acid-fast bacteria. *Nucleic acids research* **19**, 1955 (1991).
128. European Committee on Antimicrobial Susceptibility Testing (EUCAST). EUCAST Clinical Breakpoints and Dosing of Antibiotics, Version 15.0. (2025).

129. Zhou, Z.*, et al.* The EnteroBase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity. *Genome research* **30**, 138-152 (2020).
130. Joshi, N. & Fass, J. Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ files (Version 1.33)[Software]. (2011).
131. Prjibelski, A., Antipov, D., Meleshko, D., Lapidus, A. & Korobeynikov, A. Using SPAdes de novo assembler. *Current protocols in bioinformatics* **70**, e102 (2020).
132. Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows–Wheeler transform. *bioinformatics* **25**, 1754-1760 (2009).
133. Wood, D.E., Lu, J. & Langmead, B. Improved metagenomic analysis with Kraken 2. *Genome biology* **20**, 1-13 (2019).
134. Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P. & Tyson, G.W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome research* **25**, 1043-1055 (2015).
135. Seemann, T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068-2069 (2014).
136. Bayliss, S.C., Thorpe, H.A., Coyle, N.M., Sheppard, S.K. & Feil, E.J. PIRATE: A fast and scalable pangenomics toolbox for clustering diverged orthologues in bacteria. *Gigascience* **8**, giz119 (2019).
137. Mourkas, E.*, et al.* Host ecology regulates interspecies recombination in bacteria of the genus Campylobacter. *Elife* **11**, e73552 (2022).
138. Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312-1313 (2014).
139. D'Alessandro, B.*, et al.* A novel prophage identified in strains from Salmonella enterica serovar Enteritidis is a phylogenetic signature of the lineage ST-1974. *Microbial genomics* **4**, e000161 (2018).
140. Bortolaia, V.*, et al.* ResFinder 4.0 for predictions of phenotypes from genotypes. *Journal of Antimicrobial Chemotherapy* **75**, 3491-3500 (2020).
141. Camacho, C.*, et al.* BLAST+: architecture and applications. *BMC bioinformatics* **10**, 1-9 (2009).
142. Carattoli, A.*, et al.* In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrobial agents and chemotherapy* **58**, 3895-3903 (2014).
143. Jeanneau, L.*, et al.* Relative Decay of Fecal Indicator Bacteria and Human-Associated Markers: A Microcosm Study Simulating Wastewater Input into Seawater and Freshwater. *Environmental Science & Technology* **46**, 2375-2382 (2012).

144. Mark Ibekwe, A. & Lyon, S.R. Microbiological evaluation of fecal bacterial composition from surface water through aquifer sand material. *Journal of Water and Health* **6**, 411-421 (2008).
145. Levine, D.P. Vancomycin: A History. *Clinical Infectious Diseases* **42**, S5-S12 (2006).
146. Caplin, J.L., Hanlon, G.W. & Taylor, H.D. Presence of vancomycin and ampicillin‐resistant *Enterococcus faecium* of epidemic clonal complex‐17 in wastewaters from the south coast of England. *Environmental Microbiology* **10**, 885-892 (2008).
147. Chopra, I. & Roberts, M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews* **65**, 232-260 (2001).
148. Aarestrup, F.M., Agerso, Y., Gerner–Smidt, P., Madsen, M. & Jensen, L.B. Comparison of antimicrobial resistance phenotypes and resistance genes in Enterococcus faecalis and Enterococcus faecium from humans in the community, broilers, and pigs in Denmark. *Diagnostic microbiology and infectious disease* **37**, 127-137 (2000).
149. Huys, G., D'Haene, K., Collard, J.-M. & Swings, J. Prevalence and molecular characterization of tetracycline resistance in Enterococcus isolates from food. *Applied and environmental microbiology* **70**, 1555-1562 (2004).
150. Rice, L.B.*, et al.* Role of class A penicillin-binding proteins in the expression of β-lactam resistance in Enterococcus faecium. *Journal of bacteriology* **191**, 3649-3656 (2009).
151. Pietta, E., Montealegre, M.C., Roh, J.H., Cocconcelli, P.S. & Murray, B.E. Enterococcus faecium PBP5-S/R, the Missing Link between PBP5-S and PBP5-R. *Antimicrobial Agents and Chemotherapy* **58**, 6978-6981 (2014).
152. Casetta, A., Hoï, A.B., de Cespédès, G. & Horaud, T. Diversity of structures carrying the high-level gentamicin resistance gene (aac6-aph2) in Enterococcus faecalis strains isolated in France. *Antimicrobial agents and chemotherapy* **42**, 2889-2892 (1998).
153. Patterson, J.E. & Zervos, M.J. High-level gentamicin resistance in Enterococcus: microbiology, genetic basis, and epidemiology. *Reviews of infectious diseases* **12**, 644-652 (1990).
154. Sulavik, M.C., Gambino, L.F. & Miller, P.F. The MarR repressor of the multiple antibiotic resistance (mar) operon in Escherichia coli: prototypic member of a family of bacterial regulatory proteins involved in sensing phenolic compounds. *Molecular Medicine* **1**, 436-446 (1995).
155. Miller, W.R., Munita, J.M. & Arias, C.A. Mechanisms of antibiotic resistance in enterococci. *Expert review of anti-infective therapy* **12**, 1221-1236 (2014).

156. Schwan, C.L., *et al.* Genotypic and phenotypic characterization of antimicrobial resistance profiles in non-typhoidal Salmonella enterica strains isolated from Cambodian informal markets. *Frontiers in Microbiology* **12**, 711472 (2021).
157. Foti, M., *et al.* Antibiotic resistance assessment in bacteria isolated in migratory Passeriformes transiting through the Metaponto territory (Basilicata, Italy). *Avian Research* **8**, 1-11 (2017).
158. Häder, D.-P., *et al.* Effects of UV radiation on aquatic ecosystems and interactions with other environmental factors. *Photochemical & Photobiological Sciences* **14**, 108-126 (2015).
159. Adekanmbi, A.O., *et al.* Solid waste dumpsite leachate and contiguous surface water contain multidrug-resistant ESBL-producing Escherichia coli carrying Extended Spectrum β-Lactamase (ESBL) genes. *BMC microbiology* **24**, 308 (2024).
160. Day, M.J., *et al.* Diversity of STs, plasmids and ESBL genes among Escherichia coli from humans, animals and food in Germany, the Netherlands and the UK. *Journal of Antimicrobial Chemotherapy* **71**, 1178-1182 (2016).
161. Pitout, J.D., Peirano, G., Chen, L., DeVinney, R. & Matsumura, Y. Escherichia coli ST1193: following in the footsteps of E. coli ST131. *Antimicrobial Agents and Chemotherapy* **66**, e00511-00522 (2022).
162. García-Meniño, I., García, V., Lumbreras-Iglesias, P., Fernández, J. & Mora, A. Fluoroquinolone resistance in complicated urinary tract infections: Association with the increased occurrence and diversity of Escherichia coli of clonal complex 131, together with ST1193. *Frontiers in Cellular and Infection Microbiology* **14**, 1351618 (2024).
163. Manges, A.R., *et al.* Multilocus sequence typing and virulence gene profiles associated with Escherichia coli from human and animal sources. *Foodborne pathogens and disease* **12**, 302-310 (2015).
164. Rangama, S., *et al.* Mechanisms involved in the active secretion of CTX-M-15 β-lactamase by pathogenic Escherichia coli ST131. *Antimicrobial Agents and Chemotherapy* **65**, 10.1128/aac. 00663-00621 (2021).
165. Shawa, M., *et al.* Novel chromosomal insertions of IS Ecp1-bla CTX-M-15 and diverse antimicrobial resistance genes in Zambian clinical isolates of Enterobacter cloacae and Escherichia coli. *Antimicrobial Resistance & Infection Control* **10**, 1-16 (2021).
166. Fagerström, A., *et al.* Comparative distribution of extended-spectrum beta-lactamase–producing Escherichia coli from urine infections and environmental waters. *PLoS One* **14**, e0224861 (2019).
167. Alonso, C.A., González-Barrio, D., Ruiz-Fons, F., Ruiz-Ripa, L. & Torres, C. High frequency of B2 phylogroup among non-clonally related fecal Escherichia coli isolates from wild boars, including the lineage ST131. *FEMS microbiology ecology* **93**, fix016 (2017).

168. Merino, I.*, et al.* CTX-M-15-H 30Rx-ST131 subclone is one of the main causes of healthcare-associated ESBL-producing Escherichia coli bacteraemia of urinary origin in Spain. *Journal of Antimicrobial Chemotherapy* **71**, 2125-2130 (2016).
169. Chirindze, L.M.*, et al.* Faecal colonization of E. coli and Klebsiella spp. producing extended-spectrum beta-lactamases and plasmid-mediated AmpC in Mozambican university students. *BMC infectious diseases* **18**, 1-8 (2018).
170. Kiratisin, P.*, et al.* The emergence of a novel ceftazidime-resistant CTX-M extended-spectrum β-lactamase, CTX-M-55, in both community-onset and hospital-acquired infections in Thailand. *Diagnostic microbiology and infectious disease* **58**, 349-355 (2007).
171. Nguyen, V.T.*, et al.* Limited contribution of non-intensive chicken farming to ESBL-producing Escherichia coli colonization in humans in Vietnam: an epidemiological and genomic analysis. *Journal of Antimicrobial Chemotherapy* **74**, 561-570 (2019).
172. Muloi, D.*, et al.* Epidemiology of antimicrobial-resistant Escherichia coli carriage in sympatric humans and livestock in a rapidly urbanizing city. *International journal of antimicrobial agents* **54**, 531-537 (2019).
173. Bii, C.C.*, et al.* Detection of virulence-related genes by multiplex PCR in multidrug-resistant diarrhoeagenic Escherichia coli isolates from Kenya and Japan. *Epidemiology & Infection* **133**, 627-633 (2005).
174. Johnson, J.R., Owens, K., Gajewski, A. & Clabots, C. Escherichia coli colonization patterns among human household members and pets, with attention to acute urinary tract infection. *The Journal of infectious diseases* **197**, 218-224 (2008).
175. Meyer, E., Schwab, F., Schroeren-Boersch, B. & Gastmeier, P. Dramatic increase of third-generation cephalosporin-resistant E. coli in German intensive care units: secular trends in antibiotic drug use and bacterial resistance, 2001 to 2008. *Critical care* **14**, 1-9 (2010).

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