

## Genes for Multidrug Resistance in *E. coli*: One Health Challenge

### AUTHORS DETAIL

Momena Habib<sup>1\*</sup>, Aziz Ul Rahman<sup>2</sup>, Saba<sup>3</sup> Furqan Ali<sup>1</sup> and Abid Hussain<sup>1\*</sup>

<sup>1</sup>Department of Microbiology and Molecular Genetics, University of Okara, Pakistan

<sup>2</sup>Faculty of Veterinary and Animal Sciences, Muhammad Nawaz Shareef University of Agriculture, Multan Pakistan

<sup>3</sup> Department of Microbiology and Molecular Genetics, The Women University Multan, Multan, Pakistan.

\*Corresponding author: [momenakhan15@yahoo.com](mailto:momenakhan15@yahoo.com); [abid.hussain@uo.edu.pk](mailto:abid.hussain@uo.edu.pk)

Received: Sept 14, 2022 Accepted: Nov 23, 2022

### INTRODUCTION

Antimicrobial resistance (AMR) has recently been identified as one of the major public health issue affecting millions of peoples' health worldwide due to the quick evolution and spreading of resistant pathogens among humans, animals, and the environment. These antimicrobial resistant pathogens not only create hiddenness in clinician efficiency but also limits the therapeutic options which ultimately leads to increase the cost of health services by increasing infections persistence, rates of morbidity and mortality (Prestinaci et al. 2015; Morehead et al. 2018). At present it is estimated that total deaths related to AMR accounts for 700,000 annually and it is predicted to be 10 million deaths/ year by 2050. Antimicrobial resistance is becoming a complex and multifaceted threat not only for humans but also for animals and environment due to transmission of antibiotic resistance genes through movement of bacteria in different ecologies or mobile genetic elements and their acquisition by highly pathogenic bacteria in each interrelated segment (de Kraker et al. 2016).

Commensal *E. coli* usually inhabits gastrointestinal tracts of warm-blooded animals including human as part of their normal flora and is also ubiquitous in the environment. Commensal *E. coli* generally do not cause any infection to the host but harboring transmissible resistance elements have led to the evolution of this bacteria into extremely modified antibiotic-resistant pathogen and now it's becoming a challenge as infection caused by resistant patho-types are difficult to manage (Rajagopal et al. 2021). The emergence of antibiotic resistant is not restricted to *E. coli* strains

isolated from human but also reported in animal and environment origin strains. The emergence of AMR *E. coli* is mainly observed due to overuse of antibiotics in veterinary and agriculture settings (Shakya et al. 2016; Amézquita-López et al. 2019).

### MDR Emergence: Global Drug Resistance Pattern Reported Data from Animal, Human and Environment

Antimicrobials are routinely used to treat diarrhea, gastroenteritis, mastitis and urinary tract infection caused by *E. coli* in animals. However, use of antimicrobials for treatment and as a growth promoter have been associated to the development of multidrug-resistant (MDR) *E. coli* that is not only becoming a public health threat but also poses economic burden for farmers due to increase in treatment cost. These AMR bacteria and/or pathogens can be transmitted from animals to human and then spread to their environment and enter in food chain. Many studies showed the spread of antimicrobial resistant *E. coli* among chickens, humans, livestock, and respective environmental settings (Heuer et al. 2011; Novais et al. 2013; Wichmann et al. 2014; Aworh et al. 2016; Amézquita-López et al. 2019). Different studies reported that *E. coli* isolated from livestock demonstrated resistance against different classes of antimicrobials (Penicillin, macrolide, aminoglycoside and sulfonamide) (Brennan et al. 2016; Astorga et al. 2019; Azabo et al. 2022). Especially, food producing animals are becoming a potential source of dissemination of antimicrobial resistant pathogen into environment through their feces as poultry waste is routinely used for the feed of fish and shellfish (Nhung et al. 2016). Globally, different antibiotics are used in production and treatment of disease in Cattles and Buffaloes and are becoming an important reservoirs of AMR bacteria. In 2021 a study was conducted in Northeast Mexico and isolates from cattle feces and carcasses showed 72.7% overall prevalence of MDR *E. coli* (Martínez-Vázquez et al. 2021). Some other studies have also described the incidence of MDR *E. coli* as 44 %, 69%, 56% and 37% in Egypt, Portugal, France and Jordan respectively (Obaidat et al. 2018; Amador et al. 2019; Bourély et al. 2019; Ramadan et al. 2020).

A study was conducted in China and reported that extended spectrum  $\beta$ -lactamase (ESBL) *E. coli* was found in the soil that was treated with pig dung and genetic analysis of isolates from soil have showed above 90% similarity with pig dung isolates (Gao et al. 2015). Another study reported the isolation of genetically related *E. coli* from pond water and duck fecal samples (Ma J et al. 2012). Such studies indicating

**Table 1:** Antimicrobial Resistance Profiling of MDR *E. coli*.

| Country     | Strain Resistance  | Reference              |
|-------------|--|------------------------|
| Dubai, UAE  | <i>E. coli</i> Ampicillin, Tetracycline, Co-trimoxazole, Gentamicin and Enrofloxacin   | Abdelwahab et al. 2022 |
| Austria     | <i>E. coli</i> Oxacillin, Ceftazidime, Colistin, Nalidix acid, Sulfamethoxazole, Tylosin   | Hess et al. 2022       |
| Romania     | <i>E. coli</i> Ampicillin, Fosfomycin, Norfloxacin, Amoxicillin/Clavulanic Acid, Gentamicin  | Imre et al. 2022       |
| Ghana       | <i>E. coli</i> Ceftazidime, Cefuroxime, Augmentin  | Kichana et al. 2022    |
| South Korea | <i>E. coli</i> Tetracycline, Ampicillin, Ciprofloxacin, Streptomycin, Chloramphenicol  | Song et al. 2022       |
| Germany     | <i>E. coli</i> Streptomycin, Ampicillin, Amoxicillin/Clavulanic acid, Sulfonamides, tetracycline   | Gross et al. 2022      |
| Bangladesh  | <i>E. coli</i> Levofloxacin, Ciprofloxacin, Cefotaxime, Imipenem, Doxycycline  | Mandal et al. 2022     |
| Norway      | <i>E. coli</i> Ceftriaxone, Ceftazidime, Cefotaxime, Cotrimoxazole, Ampicillin, Ciprofloxacin, Pulingam et al. 2021<br>Meropenem, Imipenem |                        |

a strong relation of AMR *E. coli* transmission from animals to environment. Recently a study conducted in 2022 on sheltered dogs in Kanto region of Japan and detected MDR *E. coli* in 18 samples by detecting  $\beta$ -lactamase genes (*blaTEM*, *blaDHA-1*, *blaCTX-M-9* group and *CTX-M-14*) and quinolone-resistance protein genes (*qnrB* and *qnrS*) (Hata et al. 2022). Previous studies have reported that MDR bacteria in the cohort animals can be a health risk to humans (Shakya et al. 2016; Rajagopal et al. 2021).

### Epidemiology and Global Burden of MDR *E. coli*

Antimicrobial resistance in bacteria is one of the major public health concerns that may lead to the deaths of 10 million people annually by the end of 2050. Therefore, World Health Organization (WHO) and some other regulatory bodies have declared AMR as the most emergency condition that should be immediately addressed (Murray et al. 2022). AMR resulted in the deaths of 700,000 individuals in the year 2014 and the estimated number of deaths in different regions of the world by 2050 are given in Fig. 1. If this issue left unnoticed, circumstances may lead to the widespread emergence of extremely pathogenic and multi-drug resistant bacteria that never existed before (Pulingam et al. 2021).

According to the data retrieved from WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) report 2021, it became evident that *E. coli* and *K. pneumoniae* are the most antimicrobial resistant pathogens isolated from community, hospital, and unknown origins. MDR *E. coli* strains isolated from different regions of the world displayed resistance against many of the commonly used antibiotics and even against 3<sup>rd</sup> generation cephalosporins as summarized in Table 1.

Resistance of *E. coli* against 3<sup>rd</sup> generation cephalosporins was at maximum prevalence level of 58.3% in economically developing countries while that of 17.5% for economically developed countries (Pulingam et al. 2021). Colistin is the last resort antibiotic used against MDR *E. coli* that are even resistant against carbapenems but many different strains of *E. coli* have also developed resistance against colistin through mutations in the mobile colistin resistance (*mcr*) genes. Ten different homologues of *mcr* have been known to cause colistin resistance in MDR *E. coli* and their prevalence in different continents of the world is clearly depicted in Fig. 2 (Dadashi et al. 2021).

Low- and middle-income countries have very high prevalence of diseases involving MDR *E. coli* but due to their inadequate surveillance systems, complete data is not available (Peirano et al. 2022).

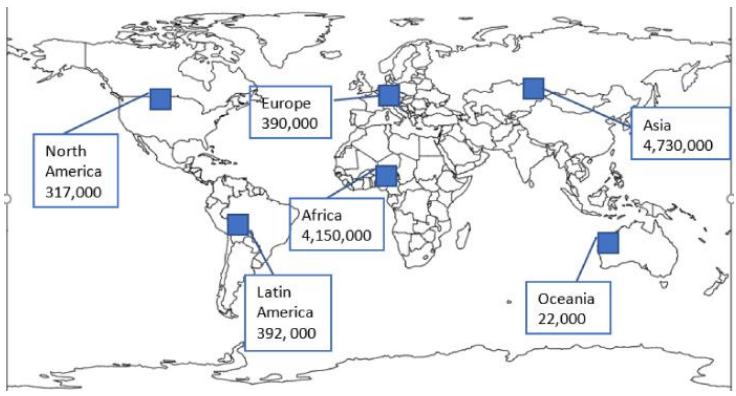
### Genetic Variability of *E. coli*

Genetic variability and diversity of *E. coli* is mainly due to genomic plasticity, a large gene pool, worldwide dissemination and ubiquity that plays an important role in making *E. coli* a priority pathogen resulting in extremely high drug resistance and virulence (Horesh et al. 2021). Majority of the *E. coli* are nonpathogenic; however, their genetic clusters are highly variable in host and country to country (Leekitcharoenphon et al. 2021). Horizontal Gene Transfer (HGT) is the major process responsible for genetic variability and it has been estimated that base composition and phylogenetic analysis of *E. coli* genomes showed 24.5% genomes were acquired through HGT (Yu et al. 2021). HGT involves the exchange of genetic material between different species through conjugation, transformation, or transduction. In addition to this, mutational variability is also one of the major factors of genomic diversity in *E. coli* (Pulingam et al. 2021). This higher genomic diversity makes *E. coli* more host and niche specific and diverse at the same time. *E. coli* phylogenetic relationship demonstrates its diversity that exhibits a wide host range including human, wild and domestic animals. *E. coli* has been reported in infections emerging from different environments and around the world with some common and diverse genes clusters (Fig. 3). Over the last 100 years *E. coli* host shift also taken place jumping from wild animals to domestic animals in Mexico. Such host shift pattern might be available throughout the world, as around the world anthropogenic activities and industrialization disturbed the natural habitat of the wild animals which resulted in such shifts (Fig. 4) (Yu et al. 2021).

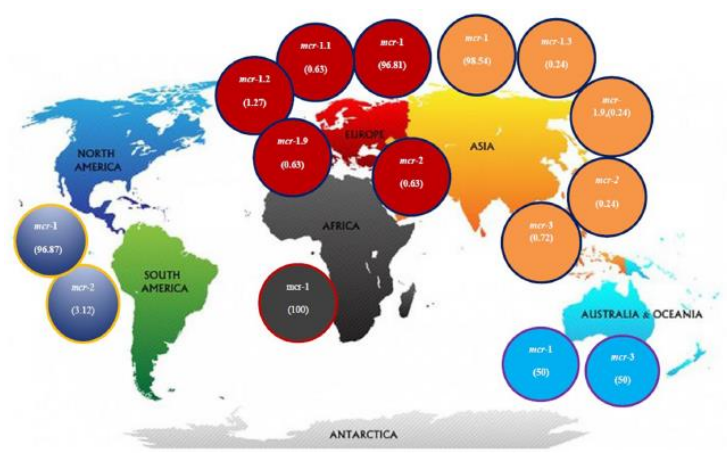
### Genes for Multi-drug Resistance in *E. coli*

Genes encoding antimicrobial resistance against different antibiotics classes have emerged in *E. coli* from both humans and animals' origins. *E. coli* exhibits extremely great genetic diversity and, antimicrobial resistance (AMR) genes in *E. coli* are located variably on chromosome and plasmids.

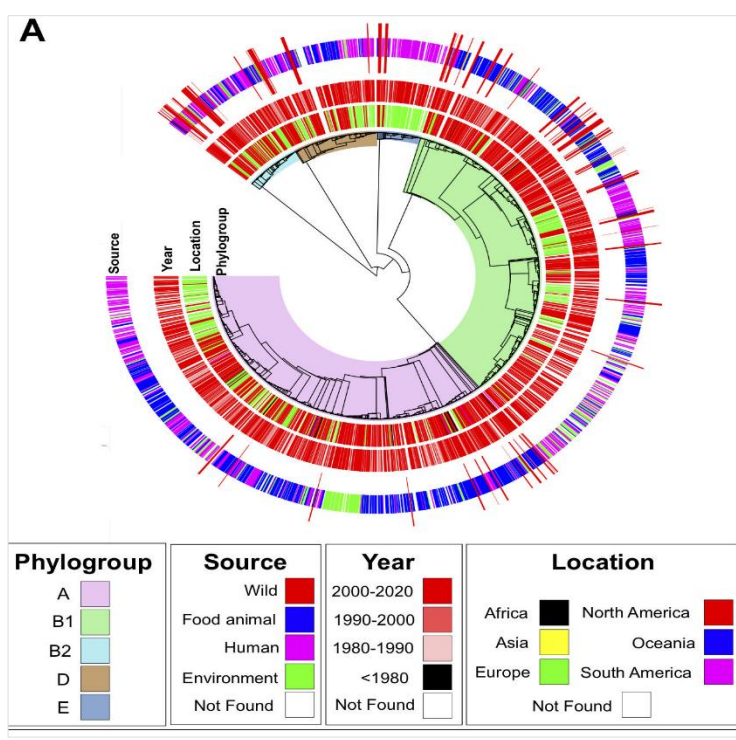
Multidrug Resistance in *E. coli*



**Fig. 1:** Estimated number of deaths in diverse areas of the world by 2050 (Pulingam et al. 2021).



**Fig. 2:** Dissemination of colistin resistant *E. coli* strains having *mcr* homologues (Dadashi et al. 2021).

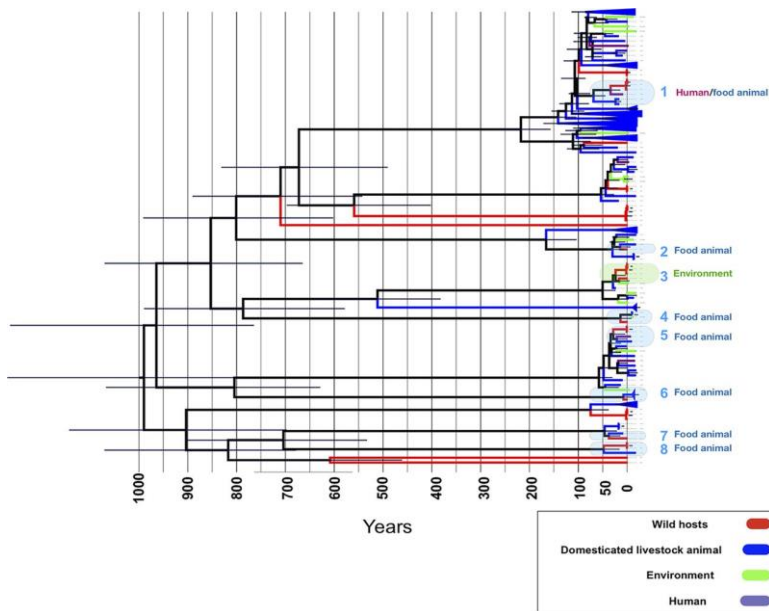


**Fig. 3:** Phylogenetic map of *E. coli* genomes isolated from different hosts, locations, and years (Murphy et al. 2021).

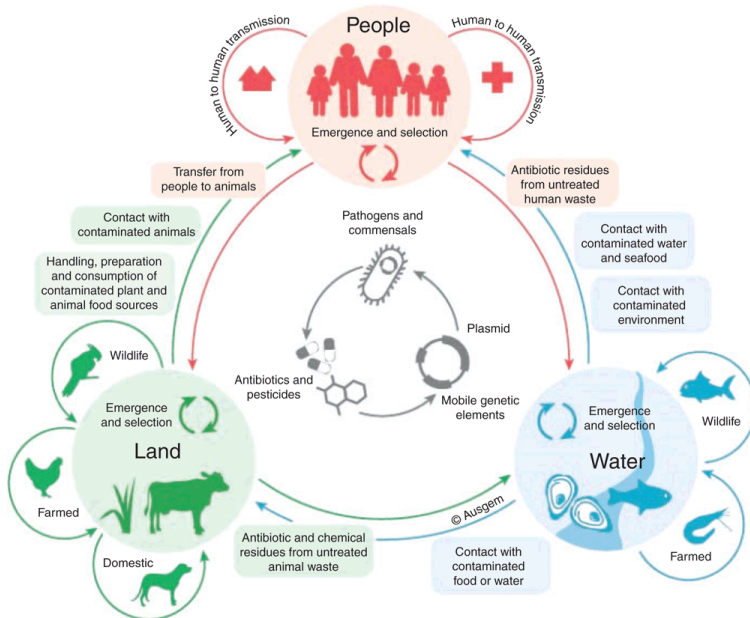
It also contains a wider variety of mobilome. Most of the AMR genes in *E. coli* are moved through different mobile genetic elements (MGEs). Intracellular transposition make

these genes mobile via plasmids and transposons. In other case integrative MGEs play role to move these genes. Some genes are also the part of integrons and move by insertion sequences.





**Fig. 4:** Phylogenetic analysis showing mixing of wild and nonwild hosts from last 100 years, which shows the *E. coli* jumping events among wild, domestic animals, human and environment (Murphy et al. 2021).



**Fig. 5:** Illustration of AMR genes transfer to Human, Animal and Environment posing severe concern for One Health (Djordjevic and Morgan 2019).

AMR genes and diverse MGEs in *E. coli* are transferred via plasmids, thus making horizontal gene transfer (HGT) a major component in AMR genes spread. Drug resistance in *E. coli* is mainly caused by mutational modifications as well as by gene transfer methods including conjugation, transformation and transduction. Spontaneous mutations account for resistance in *E. coli* against Rifampicin by altering the *rpoB* gene (Leekitcharoenphon et al. 2021, Pulingam et al. 2021). Due to the extraordinary diversity of the genome and wider host range of *E. coli*, finding new AMR genes and their spread mechanism is still under research, however a number of AMR genes around the world has been identified in vast variety of host ranges for carrying antimicrobial resistance.

### AMR Genes Mobility and One Health Challenge

Higher diversity and variability of MGEs in *E. coli* presumably makes AMR genes more vulnerable to transfer not only within species but to intraspecies normal flora, pathogens and environmental microflora and then becomes a constant challenge for human animals and environment. Multiple AMR genes harboring MDR and XDR strains have been isolated throughout the world from human, livestock, wild animals, soil, water and food. This greatly posing a concern that along with human, animal and environmental health, food security will also be a one of the major changes due to the spread of AMR in future (Djordjevic and Morgan 2019). Fig. 5 shows an illustration of AMR genes transfer through HGT and impact on One Health.

## Conclusion

*E. coli* is gram negative bacteria, having wide variety of host range, ubiquitous in environment due to flexible growth requirements and highly diverse genome. The genome of the *E. coli* harbors several AMR genes which are located on chromosome and plasmids. The presence of wide variety of mobile genetic elements makes AMR genes more vulnerable to move intracellularly and extracellularly. Due to the widespread of AMR genes globally MDR and XDR strains of *E. coli* has been emerged. Antimicrobial resistance is a rapidly expanding issue in the world. On every passing day AMR genes are being spread at an alarming rate. This spread of genes is among interspecies as well intraspecies through horizontal genes transfer and this spread posing a severe health concern for human, animal and environment.

## REFERENCES

- Abdelwahab GE et al., 2022. Antibiotics resistance in *Escherichia coli* isolated from livestock in the Emirate of Abu Dhabi, UAE, 2014–2019. *International Journal of Microbiology* 3411560: 10.
- Amézquita-López BA et al., 2016. Antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* O157 and Non-O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. *Antimicrobial Resistance and Infection Control* 5: 1- 6.
- Amador P et al., 2019. Prevalence of antibiotic resistance genes in multidrug-resistant *Enterobacteriaceae* on Portuguese livestock manure. *Antibiotics* 8: 23.
- Astorga F et al., 2019. Antimicrobial resistance in *E. coli* isolated from dairy calves and bedding material. *Heliyon* 5: e02773.
- Athanasakopoulou Z et al., 2021. Antimicrobial resistance genes in ESBL-producing *Escherichia coli* isolates from animals in Greece. *Antibiotics* 10: 389.
- Aworh MK et al., 2019. Prevalence and risk factors for multi-drug resistant *Escherichia coli* among poultry workers in the Federal Capital Territory, Abuja, Nigeria. *PloS One* 14: e0225379.
- Aworh MK et al., 2020. Extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* among humans, chickens and poultry environments in Abuja, Nigeria. *One Health Outlook* 2: 1- 11.
- Aworh MK et al., 2021. Genetic relatedness of multidrug resistant *Escherichia coli* isolated from humans, chickens and poultry environments. *Antimicrobial Resistance and Infection Control* 10: 1- 13.
- Azabo RR et al., 2022. Antimicrobial resistance pattern of *Escherichia coli* isolates from small scale dairy cattle in Dar es Salaam, Tanzania. *Animals* 12: 1853.
- Bourély C et al., 2019. Co-resistance to amoxicillin and tetracycline as an indicator of multidrug resistance in *Escherichia coli* isolates from animals. *Frontiers in Microbiology* 10: 2288.
- Brennan E et al., 2016. Multidrug-resistant *Escherichia coli* in bovine animals, Europe. *Emerging Infectious Diseases* 22: 1650.
- Dadashi M et al., 2021. Global prevalence and molecular epidemiology of mcr-mediated colistin resistance in *Escherichia coli* clinical isolates: A systematic review. *Journal of Global Antimicrobial Resistance* 29: 444- 461.
- De Kraker ME et al., 2016. Will 10 million people die a year due to antimicrobial resistance by 2050? *PLoS Medicine* 13: e1002184.
- Devanga Ragupathi NK et al., 2020. First Indian report on genome-wide comparison of multidrug-resistant *Escherichia coli* from blood stream infections. *PLoS One* 15: e0220428.
- Djordjevic SP and Morgan BS, 2019. A One Health genomic approach to antimicrobial resistance is essential for generating relevant data for a holistic assessment of the biggest threat to public health. *Microbiology Australia* 40: 73-76.
- Gao L et al., 2015. Application of swine manure on agricultural fields contributes to extended-spectrum  $\beta$ -lactamase-producing *Escherichia coli* spread in Taiwan, China. *Frontiers in Microbiology* 6: 313.
- Gross S et al., 2022. High rates of Multidrug-Resistant *Escherichia coli* in Great Cormorants (*Phalacrocorax carbo*) of the German Baltic and North Sea Coasts: Indication of environmental contamination and a potential public health risk. *Pathogens* 11: 836.
- Hess C et al., 2022. *Escherichia coli* isolated from organic laying hens reveal a high level of antimicrobial resistance despite no antimicrobial treatments. *Antibiotics* 11: 467.
- Heuer H et al., 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology* 14: 236- 243.
- Horesh G et al., 2021. A comprehensive and high-quality collection of *Escherichia coli* genomes and their genes. *Microbial Genomics* 7: 000499.
- Imre K et al., 2022. Occurrence, pathogenic potential and antimicrobial resistance of *Escherichia coli* isolated from raw milk cheese commercialized in Banat Region, Romania. *Antibiotics* 11: 721.
- Kichana E et al., 2022. Genetic characterization and antimicrobial susceptibility of *Escherichia coli* isolated from household water sources in northern Ghana. *Journal of Water and Health* 20: 771-780.
- Leekitcharoenphon P et al., 2021. Genomic evolution of antimicrobial resistance in *Escherichia coli*. *Scientific Reports* 11: 1- 12.
- Ma J et al., 2012. Characterization of extended-spectrum  $\beta$ -lactamase genes found among *Escherichia coli* isolates from duck and environmental samples obtained on a duck farm. *Applied and Environmental Microbiology* 78: 3668- 3673.
- Mandal AK et al., 2022. Epidemiology and antimicrobial resistance of *Escherichia coli* in broiler chickens, farmworkers, and farm sewage in Bangladesh. *Veterinary Medicine and Science* 8: 187-199.
- Martínez-Vázquez AV et al., 2021. Multidrug resistance of *Escherichia coli* strains isolated from Bovine Feces and Carcasses in Northeast Mexico. *Frontiers in Veterinary Science* 270.
- Morehead MS et al., 2018. Emergence of global antibiotic resistance. *Primary care: Clinics in Office Practice* 45: 467- 484.
- Murphy R et al., 2021. Genomic epidemiology and evolution of *Escherichia coli* in wild animals in Mexico. *Mosphere* 6: e00738-00720.
- Murray CJ et al., 2022. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 399: 629- 655.

- Nhung NT et al., 2016. Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: a review. *Antibiotics* 5: 37.
- Novais C et al., 2013. Spread of multidrug-resistant *Enterococcus* to animals and humans: an underestimated role for the pig farm environment. *Journal of Antimicrobial Chemotherapy* 68: 2746- 2754.
- Obaidat MM et al., 2018. Major diseases, extensive misuse, and high antimicrobial resistance of *Escherichia coli* in large-and small-scale dairy cattle farms in Jordan. *Journal of Dairy Science* 101: 2324- 2334.
- Peng JJ et al., 2021. Identification of antimicrobial resistance genes and drug resistance analysis of *Escherichia coli* in the animal farm environment. *Journal of Infection and Public Health* 14: 1788- 1795.
- Peirano G et al., 2022. Genomic epidemiology of global carbapenemase-producing *Escherichia coli*, 2015–2017. *Emerging Infectious Diseases* 28: 924.
- Prestinaci F et al., 2015. Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health* 109: 309-318.
- Pulingam T et al., 2021. Antimicrobial resistance: prevalence, economic burden, mechanisms of resistance and strategies to overcome. *European Journal of Pharmaceutical Sciences* 170: 106103.
- Ramadan H et al., 2020. Antimicrobial resistance, genetic diversity and multilocus sequence typing of *Escherichia coli* from humans, retail chicken and ground beef in Egypt. *Pathogens* 9: 357.
- Rajagopal K et al., 2021. A One Health Review of Community-Acquired Antimicrobial-Resistant *Escherichia coli* in India. *International Journal of Environmental Research and Public Health* 18: 12089.
- Shakya S et al., 2016. Occurrence and characteristics of extended-spectrum  $\beta$ -lactamases producing *Escherichia coli* in foods of animal origin and human clinical samples in Chhattisgarh, India. *Veterinary World* 9: 996.
- Song HJ et al., 2022. Antimicrobial resistance in *Escherichia coli* isolates from healthy food animals in South Korea, 2010–2020. *Microorganisms* 10: 254.
- Wichmann F et al., 2014. Diverse antibiotic resistance genes in dairy cow manure. *MBio* 5: e01017-13.
- Yu D et al., 2021. A review of the taxonomy, genetics, and biology of the genus *Escherichia* and the type species *Escherichia coli*. *Canadian Journal of Microbiology* 67: 553- 571.