

# Antibiotic Resistance in *Escherichia coli* from Animals, Food and Humans

## (Resistensi Antibiotik pada *Escherichia coli* yang berasal dari Hewan, Makanan dan Manusia)

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### ABSTRACT

Antibiotic resistance is considered a global public health problem and is related to the problem of resistance of bacteria in human and veterinary medicine that are transmitted directly and through the food chain. Uncontrolled use of antibiotics in veterinary practice is a special danger for the development of antibiotic resistance. The problem of public health, humans and veterinary is the acquired resistance of bacteria to antibiotics. Of particular importance is the emergence of multidrug resistance to *Escherichia coli*, which is becoming more common in the world, both in human and veterinary medicine, and the possible transmission of resistant *Escherichia coli* between animals and humans. The purpose of this paper is to show the importance of the rational use of antibiotics in animals and humans to prevent the spread of antibiotic resistance. *Escherichia coli* is an intestinal bacterium of all mammals, widespread in the environment and often present in food of animal origin. Today, a pluripotent bacterium and a carrier of antibiotic resistance genes due to anthropogenic factors, and genes are transmitted through animal bacteria, food bacteria to bacteria of human origin.

**Key words:** *Escherichia coli*, the pathogen of humans, animals and food, antibiotic resistance

### ABSTRAK

Resistensi antibiotik merupakan masalah kesehatan masyarakat global dan terkait dengan masalah resistensi bakteri pada manusia dan hewan yang ditularkan secara langsung maupun melalui rantai makanan. Penggunaan antibiotik yang tidak terkontrol dalam praktik kedokteran hewan sangat berbahaya khususnya bagi berkembangnya resistensi antibiotik. Masalah kesehatan masyarakat, baik pada manusia dan hewan adalah resistensi bakteri terhadap antibiotik. Munculnya resistensi multiobat terhadap *Escherichia coli*, telah umum terjadi baik pada kedokteran maupun veteriner, dan kemungkinan telah terjadi penularan *Escherichia coli* yang resisten antara hewan dan manusia. Penulisan makalah ini bertujuan untuk menunjukkan pentingnya penggunaan antibiotik yang benar pada hewan dan manusia untuk mencegah penyebaran resistensi antibiotik. *Escherichia coli* adalah bakteri usus dari semua mamalia, tersebar luas di lingkungan dan sering ditemukan dalam makanan yang berasal dari hewan. Pada saat ini, bakteri tersebut termasuk bakteri pluripoten dan pembawa gen resistensi antibiotik karena faktor antropogenik, dimana gen ditularkan melalui bakteri hewan, bakteri makanan ke bakteri asal manusia.

**Kata kunci:** *Escherichia coli*, patogen pada manusia, hewan dan makanan, resistensi antibiotik

### INTRODUCTION

*Escherichia coli* (*E. coli*) is a species of bacterium, which according to the taxonomic hierarchy belongs to the genus *Escherichia*, family *Enterobacteriaceae*, order *Enterobacteriales*, class  $\gamma$ -*Proteobacteria*, phylum *Proteobacteria* composed of over 200 genera including the genus *Escherichia*. In addition to *E. coli*, the genus *Escherichia* also includes the following species: *E. albertii*, *E. fergusonii*, *E. hermannii* and *E. marmotae*. *E. adecarboxylata*, *E. blattae* and *E. vulneris* were also considered part of the

genus but were reclassified (Euzéby 2019; Arcari et al. 2020). *Escherichia coli* is a Gram-negative, facultative anaerobic enterobacteria belonging to the colon microflora. There are apathogenic and pathogenic strains. Apathogenic strains are more dominant and are present in all mammals. Apathogenic strains are beneficial to the host organism, perform several physiological functions, and prevent intestinal colonization by various pathogens (Jang et al. 2017). Pathogens predominate in the case of disturbed, sensitive intestinal microflora and are physiologically present in the intestines in small quantities. Some

animals are often asymptomatic carriers of pathogenic strains of *E. coli*, which is transmitted to humans. Large and small ruminants are most often asymptomatic carriers of enterotoxigenic *E. coli* (ETEC) (Wang et al. 2017). This bacterium can inhabit the intestinal tract of various animals and transmission is thought to take place through a contaminated environment. The animals are practically disease-free except for young and susceptible individuals. It is a bacterium that poses a danger to humans because it belongs to food-borne zoonoses (EFSA & ECDC 2017). Beside enterotoxigenic *E. coli*, the verotoxinogen *E. coli* (VTEC) is very similar to *Shigella* disease and serovar O157: H7 is particularly dangerous. In humans, it most commonly causes gastrointestinal problems, with all the symptoms of acute food poisoning (Riley 2014). In order to prevent acute food poisoning, which is most often transmitted to humans through food, it is important to detect critical points on slaughter lines, which is necessary to reduce the risk of contamination of meat with bacteria and the risk of cross-contamination.

The use of antibiotics in veterinary and human medicine is also very widespread and antibiotics often do not achieve the desired effects because antibiotic resistance is widespread and becomes a public health problem (Stedt et al. 2014). Antibiotic resistance genes can be transmitted through bacteria from food to bacteria from the environment (Roth et al. 2019).

To ensure a quality and safe final product, it is important to apply the concept of analysis of critical points in primary production – *Hazard Analysis Critical Control Point* – HACCP, which includes detection of critical points in different stages of primary production, risk identification on slaughter lines, primary processing, etc. The application of the HACCP concept is enabled by the application of *Good Manufacturing Practice* – GMP, which includes the analysis of technological processes and guarantees technological correctness in primary production and processing. *Good Manufacturing Practice* does not guarantee a safe product for consumers, because it cannot predict possible contamination, so it is important to apply *Good Hygienic Practice* – GHP to satisfy the final product in terms of quality, hygiene and health. The HACCP system needs to be improved and the *World Health Organization* – WHO together with the *International Commission on Microbiological Specifications for Food* – ICMSF are working to improve the concept of the HACCP system to contribute to food quality, safety and health “*from farm to table*” (Osimani et al. 2013; Fan 2019).

This paper aims to show the importance of the rational use of antibiotics in animals and humans to prevent the spread of antibiotic resistance

## MORPHOLOGY AND PHYSIOLOGY OF *ESCHERICHIA COLI*

*Escherichia coli* was discovered in 1885 by paediatrician Theodor Escherich who isolated this bacterium from the faeces of a newborn child (Mainil 2013). The nonpathogenic *E. coli* belongs to the saprophytic microbiota of the intestinal tract of humans and animals (Leimbach et al. 2013). Given its persistence in the intestinal tract, it is an indicator of faecal contamination of water, food, and ecosystems in general (Zannoto et al. 2016). It is usually resistant and can live for months in water, soil, objects, food (Blount 2015). *E. coli* is a Gram-negative bacillus with rounded or straight ends 2-6 µm long, 1.1-1.5 µm in diameter, with possible variations depending on the corresponding strain. The schedule is single or in pairs (Blount 2015; Maksimović & Rifatbegović 2015). Like many of the *Enterobacteraceae* family, *E. coli* is anaerobic, enzymatic, catalase and oxidase-positive and reduces nitrate to nitrite (Table 1) (Markey et al. 2013).

Some strains possess a capsule. The motility of *E. coli* is conditioned by the possession of flagella, and immobile strains contain fimbriae that allow the bacterium to adhere to another cell (Blount 2015). Some strains are hemolytic. Due to its ability to ferment lactose, it changes the colour of the substrate (MacConkey agar-pink, blue agar-yellow). Colonies are usually typed S with the possibility of forming R and M colonies (Islam et al. 2014; Maksimović & Rifatbegović 2015).

**Table 1.** Biochemical tests for *E. coli*

| Tests                     | Results                       |
|---------------------------|-------------------------------|
| Indole (tryptophan broth) | +                             |
| Methyl red (MR)           | +                             |
| Voges proskauer (VP)      | -                             |
| Citrate (citrate agar)    | -                             |
| Triple sugar (TSI)        | Lactose fermentation with gas |
| Fast urease (RUT)         | -                             |
| Ortonitrophenol (ONPG)    | +                             |

**Note:** (+) = 90% and more strains positive; (-) = 90% and more strains negative

**Source:** Markey et al. (2013)

## MECHANISMS OF PATHOGENICITY OF *ESCHERICHIA COLI*

The pathogenicity of *E. coli* is related to its ability to adhere, motility, the ability to build sessile communities, toxicity, which manifests during life through exotoxins and after “*post mortem*” through endotoxins. It can possess siderophores, capsule,

hemolysins and invasins (Mageiros et al. 2021). Capsular polysaccharides of certain *E. coli* strains inhibit phagocyte activity. Capsule material also affects the antibacterial efficacy of the complement system. The release of endotoxin (cell wall lipopolysaccharide gram-negative bacteria) occurs after the death of the bacterium. Endotoxin is a pyrogenic substance, which damages the endothelium and leads to endotoxic shock (Mueller & Tainter 2022). The pathogen *E. coli* can adhere, adhering to the walls of the epithelium with the help of fimbrial adhesins, whose receptors are located along the epithelium of the urinary and intestinal tract. The most significant adhesins of *E. coli* strains, which cause diseases in domestic animals, are considered to be K88 (F4), K99 (F5), 987P (F6), F18, and F41 (Quinn et al. 2011). Fimbrial (F) antigens serve as adhesins in the binding of *E. coli* to intestinal cells (Markey et al. 2013). Pathogenic effects also result from the production of enterotoxins, Shiga-toxins or Verotoxins or through cytotoxic necrotizing factors. Unlike enterotoxins, which affect only enterocyte activity, Shiga toxins and cytotoxic necrotizing factors can lead to significant cell damage (Mueller & Tainter 2022). *E. coli* enterotoxinogen (ETEC) contains a gene for toxins and the ability to attach to the intestinal wall with the help of the enzyme adhesin (Dubreuil 2013). The same enzyme, together with the released enterotoxin, can encode genes on plasmids, which are transmitted by conjugation to another bacterium. Enterotoxin causes the movement of water from the tissue into the intestinal lumen and leads to symptoms of osmotic diarrhoea, the so-called travel diarrhoea. There are two exotoxins: thermolabile LT (60°C / 30 min.) and thermostable ST (it is not destroyed even at 100°C / 30 min.) (Prescot et al. 2005; Loos et al. 2012). The toxins cause the activity of adenyl cyclase, ie guanyl cyclase, under the action of which cyclic adenosine monophosphate (cAMP) and cyclic guanosine triphosphate (cGMP) are produced, which stimulate the secretion and at the same time block ion resorption from the intestinal lumen, leading to watery diarrhoea symptoms (Dubreuil et al. 2016). Enterotoxigenic, thermostable toxin 1 has been found in some enterotoxigenic and enteropathogenic strains and in all enterohemorrhagic strains of *E. coli*. Vero or Shiga toxin, produced by the toxicogenic *E. coli*, causes alimentary intoxications (Quinn et al. 2011).

## ESCHERICHIA COLI INFECTIONS IN ANIMALS

*Escherichia coli*, which is considered nonpathogenic, can cause opportunistic infections (Markey et al. 2013). The pathogen *E. coli* can cause

infections of various organs: urethritis, cystitis, pyelonephritis, gastroenteritis, cholecystitis, cholangitis, appendicitis, peritonitis, meningitis, metritis, pyometra, mastitis, sepsis, septicemia, and infections of the skin, soft tissues and wounds (Yassin et al. 2017). Strains of *E. coli* that cause extraintestinal infections frequently colonize the intestinal tract of all animals, whereas strains that cause enterocolitis are usually not part of the normal flora of healthy animals. Infections occur by ingestion through contaminated water, food, or direct contact with clinically or subclinically infected animals (Quinn et al. 2011). Predisposing factors for the development of clinical disease are colonization, susceptibility, immune status, age, diet and exposure of the animal to pathogenic strains. Although the division cannot be strict, *E. coli* strains can be divided into those that cause intestinal and extraintestinal infections (Table 2) (Hrustemović et al. 2021). Strains associated with extraintestinal diseases are: avian pathogens (APEC), septicemic (SEPEC), uropathogenic *E. coli* (UPEC), and strains of *E. coli*, which cause local infections (Quinn et al. 2011). Intestinal diseases are caused by: enterotoxinogen (ETEC), enteroaggregative (EAaggEC), attaching and effacing, attachment and removal of *E. coli* (AEEC), which includes enteropathogens (EPEC) and Vero or Shiga toxic strains (VTEC or STEC). Enterohemorrhagic *E. coli* (EHEC) and strains causing oedema are STEC subgroups. Unlike ETEC strains, extraintestinal pathogens, EPECs are part of the normal flora of animals and are considered conditionally pathogenic (Markey et al. 2013). *E. coli* in the case of bacteremia can reach various tissues, organs, and in some cases can cause death. The zoonotic potential of VTEC is often associated with birds (Saviolli et al. 2016). All wild animals can be carriers of pathogenic species of *E. coli*, including the hemolytic strain *E. coli* O157: H7, of zoonotic character, which secretes the so-called Shiga or Verotoxin. The pathogenesis of the disease in wild animals has not been described. The intestinal tract of animals is colonized by the same serotype of *E. coli* that originates from the environment, and wild animals are significant carriers (Rice et al. 2013).

Enterotoxigenic *Escherichia coli* (ETEC) is the cause of most neonatal colibacillosis in calves, lambs, and piglets (Markey et al. 2013). Certain ETEC can become endemic on domestic animal farms and cause mass morbidities and mortality of a large number of young animals for a long time. Severe diarrhoeal forms of the disease occur in young calves. The bacterium usually enters through the nasopharynx and leads to clinical manifestations in the form of white diarrhoea.

**Table 2.** Animal diseases caused by *Escherichia coli*

| Animals                         | Illness                                                                                                                                                                       |
|---------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                 | Intestinal and extraintestinal colibacillosis                                                                                                                                 |
| Cattle                          |                                                                                                                                                                               |
| Calves up to one week of age    | "White diarrhoea" colibacillosis. Colisepticemia (sudden death due to endotoxic shock)                                                                                        |
| Calves overcame colisepticemia  | Joint disease                                                                                                                                                                 |
| Dairy cows after parturition    | Coli mastitis (peracute disease, death due to endotoxic shock)                                                                                                                |
| Sheep                           | Coli mastitis (a peracute disease similar to bovine mastitis)                                                                                                                 |
| Neonatal lamb                   | Colibacillosis and colisepticemia. "Watery mouth" (death can occur in 6-24 hours), associated with <i>E. coli</i> endotoxemia                                                 |
| Pigs                            |                                                                                                                                                                               |
| Piglets up to one week of age   | Neonatal diarrhoea (colibacillosis), mortality 90-100%. Colisepticemia.<br>Piglet meningitis (occasional septicemia with invasive strains and death within 48 hours of birth) |
| Piglets two weeks after weaning | Colibacillosis (lower mortality than in neonatal piglets)                                                                                                                     |
| Rejected piglets                | Oedema disease (sudden death, oedema, nervous symptoms)                                                                                                                       |
| Sows after farrowing            | Coli mastitis                                                                                                                                                                 |
| Colds after pollination         | Mastitis-metritis-agalactia (MMA) syndrome                                                                                                                                    |
| Dog                             | Urinary tract infections (most common bitch cystitis)                                                                                                                         |
| Neonatal puppies                | Colisepticemia (often fatal)                                                                                                                                                  |
| Bitch                           | Pyometra                                                                                                                                                                      |
| Poultry                         |                                                                                                                                                                               |
| Chickens                        | Omphalitis, yolk sac infection                                                                                                                                                |
| All age groups                  | Colisepticemia (primary or secondary infection of the intestine or respiratory tract).<br>Coligranuloma (chronic condition, often after colisepticemia)                       |

**Source:** Hrustemović et al. (2021)

The disease is often sudden, which is attributed to the strong virulence of the causative agent, the susceptibility of the attacked organism, the strength of the exotoxin and can lead to collapse and death. Calves are reservoirs of ETEC, which has zoonotic potential, meaning it is transmitted from animals to humans (Labro & Bryskier 2014; Kolenda et al. 2015). Enterotoxigenic *Escherichia coli* in newborn piglets is the cause of severe diarrhoea. Piglets can die in the first twelve hours of life. If they survive the first stage of the disease, in one to two days, watery diarrhoea occurs. Enterotoxigenic *Escherichia coli* can rapidly reproduce and produce large amounts of exotoxins within the intestinal lumen, resulting in the appearance of so-called diarrhoeal forms of the disease. In case of penetration into parenchymal organs, it can cause sepsis. The septicemic form of the disease can occur in the first week of the disease, but also somewhat later (Ahmed et al. 2013). Older domestic animals, especially cattle and pigs, are considered asymptomatic carriers of ETEC and represent a reservoir of infection for humans, other animals, and the environment (Labro & Bryskier 2014).

Typical EPEC strains were first described as a cause of neonatal diarrhoea and these strains are strictly pathogenic to humans. Atypical EPEC causes diarrhoea in calves, lambs, piglets, and puppies (Markey et al. 2013). It damages the microvilli of enterocytes and shows the greatest capacity in biofilm formation. Biofilm-coated bacteria cause chronic, persistent, and recurrent infections, due to their resistance to antibiotics and the immune system of the attacked host (Schiebel et al. 2017). Swine oedema is most commonly associated with *E. coli* strains O139 and O141, which are usually hemolytic and produce Shiga toxin, similar in activity to Shiga toxin *Shigella* species. This similarity is attributed to horizontal gene transfer via plasmids, between *Shigella* and *E. coli* (Delannoy et al. 2017). The mentioned strains of *E. coli* are physiologically inhabited in the large intestines of pigs, but risk factors, such as stress, altered diet, poor zoo hygiene, lead to accelerated reproduction of the pathogen. The same strains are sometimes associated with diseases, such as hemorrhagic enteritis in calves (Markey et al. 2013). Verotoxins cause severe mucosal damage and cause hemorrhagic colitis. *E. coli* O157:

H7 and other enterohemorrhagic serotypes have also been identified as major zoonotic agents, which are transmitted by food to humans (Rani et al. 2021). The main reservoirs of the causative agent are animals, but also reservoirs of antimicrobial resistance (Labro & Bryskier 2014).

## ANTIBIOTICS AND MECHANISMS OF ACTION

The first antibiotic (AB) discovered was penicillin, a narrower spectrum of action, secreted by the fungus *Penicillium notatum*. It was discovered in 1928 by Alexander Fleming (Fejzuli et al. 2018). The first sulfonamide was discovered in 1935, and in 1943, the first broad-spectrum antibiotic streptomycin, a member of the aminoglycoside class, was discovered (Rocha et al. 2021). Antibiotics are drugs that have a selective effect, and their mechanism of action is manifested in small concentrations, without toxic effects on host cells. We distinguish between bacteriostatic and bactericides (Arenz & Wilson 2016). Bacteriostatic inhibit bacteria, rely on the host's immune response, while bactericides cause bacterial death (Radlinski & Conlon 2018). Antibiotics can inhibit cell wall synthesis, nucleic acids, proteins, disrupt cell membrane function (Table 3) (Quinn et al. 2011). In a study in Germany, the AB most commonly used in veterinary medicine are fluoroquinolones. They

are most commonly used in the eradication of colibacillosis (Markey et al. 2013; Wallmann et al. 2016).

## METHODS FOR PROVING ANTIBIOTIC RESISTANCE

The basic methods of testing the susceptibility of bacterial isolates to antibiotics (AB) are disk diffusion, broth dilution, and agar dilution (Markey et al. 2013). Quantitative methods (broth dilution and agar dilution method) allow the measurement of the minimum inhibitory concentration (MIC) of AB (the highest dilution of antibiotics that inhibits bacterial growth). The MIC can also be determined using an E-test (Quinn et al. 2011).

The most commonly used is disk diffusion method. This method determines the bacterial susceptibility to AB, with the help of selected discs impregnated with an AB of known concentration, which is applied to the substrate on which the test culture was previously applied.

The choice of AB depends on the bacterial species being tested. Of the media, *Mueller-Hinton* agar and blood agar are most commonly used, and for some bacterial species, appropriate media additives are used (Markey et al. 2013). Diffusion of the AB on the medium creates a zone saturated with the AB, within

**Table 3.** Antibiotics and their mechanisms of action

| Antibiotic classes and sulfonamides | Mechanisms of actions                                                                                                                      |
|-------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| $\beta$ lactams                     | Inhibition of cell wall synthesis (Bactericidal action).                                                                                   |
| Glycopeptides                       | Inhibition of cell wall synthesis (Bactericidal action).                                                                                   |
| Polypeptides                        | Inhibition of cell wall synthesis (Bactericidal action)                                                                                    |
| Nitrofurans                         | Inhibition of protein synthesis Blocking 30S ribosomal activity (Bacteriostatic action).                                                   |
| Aminoglycosides                     | Inhibition of protein synthesis Blocking 30S ribosomal activity (Bactericidal action).                                                     |
| Tetracyclines                       | Inhibition of protein synthesis Blocking 30S ribosomal activity (Bacteriostatic action).                                                   |
| Lincosamides                        | Inhibition of protein synthesis. Blocking 50S ribosomal activity.(Bactericidal./ bacteriostatic)                                           |
| Macrolides                          | Inhibition of protein synthesis Blocking 50S ribosomal activity (Bacteriostatic action).                                                   |
| Quinolones/ Fluoroquinolones        | Inhibition of nucleic acid synthesis by blocking DNA gyrase (Bactericidal action).                                                         |
| Novobiocin                          | Inhibition of nucleic acid synthesis by blocking DNA gyrase (Bactericidal/ bacteriostatic).                                                |
| Rifampin                            | Inhibition of nucleic acid synthesis by blocking DNA – directed RNA polymerase (Bacteriostatic action).                                    |
| Sulfonamides                        | Inhibition of DNA synthesis by inhibition of folic acid metamolysis.<br>Antimetabolite – sulfonamide (Bacteriostatic action).              |
| Trimethoprim                        | Inhibitions of DNA synthesis by coupling with the enzyme dihydrofolate reductase<br>Antimetabolite – trimethoprim (Bacteriostatic action). |
| Nitroimidazoles                     | Inhibition of DNA replication, by blocking DNA gyrase (Bactericidal action).                                                               |

**Source:** Quinn et al. (2011)

which the isolate sensitive to that AB will not grow. The area around the AB disc, where there is no bacterial growth, is called the inhibition zone. The diameter of the inhibition zone is measured through the middle of the disc and expressed in mm. The results are compared with the standards for the interpretation of the inhibition zone. Depending on the created zone of inhibition, which is expressed in millimetres and measured by a standardized method, we conclude whether the bacterium is sensitive (S), moderately sensitive (I), or resistant (R) to the tested AB (EUCAST 2017b; CLSI 2018).

If the test bacterium is sensitive to a particular AB, the AB is usable in the usual doses. When the bacterium is moderately sensitive, the AB is usable in the maximum allowable doses. If the bacterium has shown resistance to a particular AB, the AB is unusable (Quinn et al. 2011; Markey et al. 2013). Recommendations from the *European Commission for Antimicrobial Susceptibility Testing* and the *Institute for Clinical and Laboratory Standards* (EUCAST 2017a; EUCAST 2017b; CLSI 2018) are used for testing procedures, AB selection, and interpretation of results. Significant changes in the zone of inhibition affect the interpretation of the antimicrobial susceptibility (AMS), so it is important not to deviate from the standard recommendations for the use of the disk diffusion method.

Factors that may affect the size of the inhibition zone are incubation conditions, bacterial inoculum, medium composition, content, age, storage of tested antibiotics, inoculation method, test culture, method of reading the results (Quinn et al. 2011). There are also innovative phenotypic tests to demonstrate different mechanisms of *E. coli* resistance. Extended-spectrum  $\beta$ -lactamases (ESBLs) and  $\beta$ -lactamases AmpC, which inactivate newer  $\beta$ -lactams, generation III cephalosporins, and carbapenems, are the most commonly detected, causing the loss of porin bacterial cells, through which AB enters the bacterium to exert their effects (Wilson & Török 2018). Phenotypic tests include a modified Hodge test, a combined disk test, synergism test, biochemical tests, and spectrophotometry. More recently, a molecular PCR method has been used to detect antibiotic resistance genes, which encode various types of extended-spectrum  $\beta$ -lactamases, ESBLs, and clones (Lee et al. 2013; Osei Sekyere et al. 2015; Hrustemović et al. 2021).

## PROBLEMS OF ANTIBIOTIC RESISTANCE OF BACTERIA

Antibiotics (AB) are effective drugs, which are among the most commonly prescribed drugs, but bacteria sooner or later develop various types of

resistance mechanisms, which are related to the mechanism of action of AB (Hendriksen et al. 2008). Alexander Fleming also warned about the possibility of antimicrobial resistance (AMR) in the case of irrational use of AB, which has come true today and AMR is a public health problem (Hogberg et al. 2010). Based on global data, 50% of the total number of detected AB is used in veterinary medicine to express the preventive and therapeutic effects in animals, which are used for human consumption. A particular problem is the irrational use of AB, where opportunistic bacteria become reservoirs of resistance genes (Hrustemović et al. 2021).

Animals transmit resistance genes to humans through the food chain (Szmolka & Nagy 2013). Antibiotics can exhibit a cumulative effect on the individual organism. Residues can be found in meat, milk, eggs and other products of animal origin. Harmful consequences are reflected in environmental pollution and the harmful effects of AB residues on animals and humans, who consume meat and products of animal origin. The use of AB changes the composition of the microflora and the irrational use of AB increases the risk of creating AMR, autoimmune diseases, allergic syndromes, etc. (Meng et al. 2018; De Martinis et al. 2020; Hrustemović et al. 2021). The problem with the use of broad-spectrum AB is the selection of resistant bacteria, which show mutations caused by variation in hereditary material (genome) and can be transmitted to offspring and different bacteria by the horizontal transmission of resistance genes (Cantas et al. 2013).

Selected bacteria easily multiply in the macroorganism when using AB (Kalenić et al. 2013). Resistance genes can also be transmitted to nonpathogenic bacteria (Dantas & Sommer 2014). In veterinary medicine, AB, in addition to exhibiting a therapeutic effect, can exert the effects of biostimulation, which are used in animals for food production (Dafale et al. 2020). The use of bio stimulators reduces the incidence of disease in animals and contributes to food safety in production. However, in recent times there is evidence to suggest the risk of antimicrobial resistance due to irrational use of AB and thus the European Union has banned the use of AB in animals as biostimulants as a measure to protect against AMR (Chantziaras et al. 2014; Hao et al. 2014).

International trade in beef and poultry has contributed to the spread of resistance mediated by plasmid and other extended-spectrum  $\beta$ -lactamases, CTX-M-14 and clones, often responsible for reduced susceptibility to carbapenems (Alonso et al. 2017). Carbapenemase-producing bacteria are usually resistant to all existing antibiotics (Tängdén & Giske 2015).

Consumption of heat-treated food of animal origin, which contains AB residues, is also a danger to

public health. Many chemical structures during heat treatment can become even more toxic and cause several harmful effects on the individual organism (Bacanli & Başaran 2019). Due to the consumption of different AB during life, bacteria, especially commensals, acquire different resistance genes or develop resistant mutants, all for bacterial survival and maintenance of homeostasis in the intestinal tract (Boireau et al. 2017).

Bacteria are short-lived, variable genetic material, which changes frequently (acquired resistance), depending on the use of certain antibiotics, which allows them to acquire various mechanisms of antibiotic resistance (Szmolka & Nagy 2013; Boireau et al. 2017). Thanks to their acquired mechanisms of resistance, without changing their genetic and phenotypic characteristics, bacteria can possess antibiotic resistance (ABR) for a long time and in the time of abstinence from AB therapy (Richardson 2017). ABR can be innate and acquired. Congenital bacterial resistance is a genetic characteristic of a particular bacterium. It occurs due to a mutation in the bacterial genome or due to the acquisition of a new deoxyribonucleic acid, creating a new mechanism of resistance (Poirel et al. 2018).

The problem of public health, human and veterinary medicine is the acquired ABR of bacteria, which begins to change uncontrollably (Poirel et al. 2018). Acquired bacterial resistance represents an unpredictable genetic change in the bacterium, due to which it acquires resistance to an antibiotic that is effective in ancestors, ineffective in offspring. A change in the genetic material of a bacterium occurs when it is found in unfavourable living conditions, such as the use of AB, or when it tries to survive. Mutations lead to changes in the genetic material of the bacterium (Boireau et al. 2017). The causes of mutations, errors in the bacterial genome, can be spontaneous and induced. Spontaneous mutations are the result of a DNA fission error, while induced ones are caused by the action of physical and chemical mutagens such as an antibiotic (Pavlica 2012). An example of an AMR in the form of a mutation is the resistance of the bacterium to isoniazid.

Bacteria can change their hereditary material through the exchange of DNA between different microorganisms, including those of different phylogenetic affiliation, originating from different animals and humans. The phenomenon is called horizontal gene transfer which takes place asexually via plasmids, transposons, and gene cassettes and the process of conjugation, transformation and transduction. Receiving hereditary material from other bacteria is not based on selection according to the species, and for that reason, a new genetic strain of the

bacterium can be obtained (Poirel et al. 2018; Law et al. 2021).

Biochemical mechanisms of AMR are enzymatic change and modification, change of DNA gyrase and topoisomerase IV, target modification (change of target enzyme), change of ribosome structure, active efflux, change of metabolic pathway, change of cell membrane permeability (Poirel et al. 2018). Enzyme modification is a bacterial resistance mechanism where bacteria produce enzymes for AB inactivation. This type of created bacterial mechanism for the development of ABR has been reported with the use of  $\beta$  lactam antibiotics, such as penicillins, cephalosporins and rifamycins. Today, more than 1,300 bacterial  $\beta$ -lactamase enzymes are known to inactivate by their ability to cleave AB  $\beta$ -lactam ring linkages, where the active penicillin nucleus is six amino penicillin acids in which a five-membered thiazolidine ring is attached to the  $\beta$ -lactam ring (Szmolka & Nagy 2013). One of the enzymes produced by cephalosporin-resistant bacteria is serine  $\beta$  lactamase. The enzyme produced by resistant strains of carbapenems, which along with other mechanisms of bacterial resistance to carbapenems causes their inactivation, is carbapenem oxacillinase (OXA), responsible for the emergence of resistance of *Acinetobacter baumannii* (Shaikh et al. 2015; Poirel et al. 2018).

Gram-negative bacteria produce various  $\beta$ -lactamases, cephalosporinases, broad-spectrum and extended-spectrum  $\beta$ -lactamases which are responsible for the so-called nosocomial infections caused by multidrug-resistant bacteria which are resistant to almost all existing AB (Bush & Bradford 2020). Another mechanism of ABR of DNA gyrase and topoisomerase IV occurs when AB is used, the mechanism of action of which is based on the target sites of the DNA gyrase and topoisomerase IV subunit, which is the case with quinolones, which are often used in human and veterinary medicine in eradication *E. coli*. Deoxyribonucleic acid is located in the regions of division and non-division in the form of super threads. The form is maintained by topoisomerase IV. Excess superfilament is removed by DNA gyrase during replication. After DNA replication, topoisomerase IV helps separate daughter DNA (Hooper & Jacoby 2016; Poirel et al. 2018; Hirsch & Klostermeier 2021).

Resistance is conditioned by the appearance of mutations in genes encoding gyrase and topoisomerase IV (Poirel et al. 2018). Target modification, or alteration of the target enzyme, is a mechanism of ABR evident in gram-positive cocci, such as *Enterococcus* spp., which is based on a modification of the target binding site and the formation of a weakly binding bacterial protein. A  $\beta$ -lactam AB is usually bound to the binding protein to exert a bactericidal effect. Bacteria produce a binding protein (PBPs), which

catalyzes the synthesis of peptidoglycans. The effectiveness of AB is related to the strong affinity of the bacterial protein for AB. Decreased affinity leads to the emergence of ABR of the bacterium (Hugonnet et al. 2016; Lopatkin et al. 2021). Alteration of ribosome structure is a mechanism of ABR where the bacterial cell can acquire a gene for resistance to aminoglycosides, lincosamides, macrolides, which changes the structure of ribosome receptors, through which these antibiotics have a detrimental effect on the bacterial cell.

The inability to bind AB to the 30S and 50S subunits, bacterial ribosomal receptors, and the inability to achieve a germicidal effect is achieved through the methylase genes responsible for erythromycin resistance (Tsai et al. 2014; Martínez et al. 2018). Active efflux, as a mechanism for the development of antimicrobial resistance, is used by many gram-negative bacteria. For it to function, the bacterium must have a functional metabolism, because it takes place with energy consumption (Oliveira & Reygaert 2022). Resistant bacteria, which use active efflux as a mechanism of ABR, also achieve this by eliminating antibiotics via a pump or via a carrier protein (Szmolka & Nagy 2013). Metabolic pathway change is a mechanism of resistance where bacteria undergo metabolic changes. They develop target sites by which they use growth factors and as such show resistance to certain antibiotics.

Bacteria get the role of a native protein, thus surviving. This resistance mechanism is used by bacteria resistant to trimethoprim and sulfonamides. The mechanism is based on a decrease in bacterial sensitivity and a decrease in the affinity of the enzymes dihydropteroate synthetase and dihydropteroate reductase (Lauxen et al. 2021). Alteration of cell membrane permeability is a bacterial mechanism of resistance, which often occurs in gram-negative bacteria to certain water-soluble AB. Carbapenems achieve their mechanism of action by passing through bacterial porins (channels). This type of resistance mechanism is acquired by *Pseudomonas aeruginosa* in all AB classes (Li et al. 2012). *E. coli* is one of the first bacteria to show resistance to once-effective AB and is often multidrug-resistant (Qekwana et al. 2017; Hrustemović et al. 2021).

### **The role of livestock in the development and transmission of antibiotic resistance of *Escherichia coli* to the human population**

Antibiotic resistance is recorded as a growing trend globally including in Bosnia and Herzegovina. It requires constant monitoring and control to reduce the spread of antibiotic resistance which is a public health problem. Based on data from various scientific papers,

the spread of multidrug-resistant *E. coli* is on the rise (Jelesić et al. 2011; Yassin et al. 2017). Multidrug-resistant *E. coli* is multidrug-resistant and its resistance is caused by antibiotic resistance genes that are transmitted vertically from parents to offspring and horizontally to all bacteria in the immediate vicinity. Transmission is enabled via plasmids. Genes can also be transmitted to other strains regardless of the type and origin of *E. coli*, which also means through animal bacteria, food contaminating bacteria to human bacteria (Poirel et al. 2018). *E. coli* is a pluripotent bacterium, which means that if one type of antibiotic causes resistance in the bacterium, resistance will be present to other types of antibiotics from the same class (Jelesić et al. 2011). In the treatment of colibacillosis, and especially in urinary tract infections, fluoroquinolones are most commonly used, to which an increase in antibiotic resistance has been reported (Markey et al. 2013). B-lactam antibiotics are also used to treat colibacillosis, and an increase in antibiotic resistance has also been reported. The biochemical mechanism produced by *E. coli* to defend against this class of antibiotics is enzymatic alteration and modification or production of  $\beta$  lactamases and penicillin amidase (Poirel et al. 2018).

It is important to mention that antibiotic resistance is also recorded in wild animals, which indicates the influence of anthropogenic factors because they are not prescribed antibiotics. Antibiotic resistance can be linked to the transfer of resistance genes through ecosystems, the food chain and other sources. Transmissible resistance genes encode different resistance enzymes or carry a genetic predisposition to create biochemical mechanisms of resistance (Poirel et al. 2018). The spread of antibiotic resistance to new  $\beta$ -lactams such as carbapenems, which are the last line of defence in bacterial infections, poses a great danger to public health (Palacios et al. 2018). Resistance has been reported to old cephalosporins such as cephalothin and this has long been known. The problem is the spread of antibiotic resistance to third-generation cephalosporins used in severe, life-threatening diseases (OIE 2019). As for aminoglycosides that are also often used in veterinary and human medicine, the greatest resistance is usually present to older aminoglycosides that have been used for a long time, and such is e.g. neomycin, which is most commonly used to exert a local effect due to toxicity (Quinn et al. 2011; De Briyne et al. 2014). Neomycin, an antibiotic of the aminoglycoside class, is widely used in the treatment of conjunctivitis, wounds, dermatitis, vaginitis, mastitis, metritis, as a disinfectant for the intestinal tract, in food production as a supplement, in primary production of broiler chickens, in poultry due to increased growth in production, livestock, aquaculture, etc. therefore, neomycin resistance is widespread

(Fejzuli et al. 2018). A review of the literature showed less resistance to gentamicin, which also belongs to the class of aminoglycosides. Gentamicin is commonly used as an intramuscular antibiotic and as such is often used in clinical practice. Decreased sensitivity to this drug has been reported, which may be associated with frequent use, but the use of a higher dose gives results. In case of reduced sensitivity, antibiotic rationalization is also recommended (Hrustemović et al. 2021).

Resistance to sulfonamides is also observed in *E. coli* of various animals, but more in cattle and horses than e.g. in cats, because they are more often used in cattle in the treatment of enteritis, metritis, endometritis, retention of the second, etc. They are rarely used in systemic infections: they have been replaced by more effective drugs (Talan et al. 2000). Trimethoprim/sulfamethoxazole was once the first drug of choice in the treatment of urinary tract infections with *E. coli* and amoxicillin has also been widely used (Oelschlaeger et al. 2002). The use of monotherapy in the treatment of *E. coli* is recommended to prevent resistance and the use of bactericides with bactericides, and bacteriostatic with bacteriostatic to achieve synergism (Kristich et al. 2014). A review of various literature concludes that antibiotics must be used rationally in veterinary medicine, but also agriculture due to the spread of resistance genes through the food chain. The efficacy of the bactericidal effect of various antibiotics on *E. coli* is variable and empirical therapy is not recommended. Uncontrolled use of antibiotics also disrupts the socio-economic situation of a country, because antibiotics are expensive drugs (Zoretić 2016). Antibiotics are the most commonly prescribed drugs in the world (Kalenić 2013). The invention of antibiotics has prolonged life and they must not be lost.

Numerous studies suggest that the rational use of antibiotics reduces the spread of antibiotic resistance (Wasył et al. 2018). Rzewuska et al. (2015) from Poland examined the state of antibiotic resistance of intestinal and extraintestinal *E. coli* isolates from dogs and cats by examining the resistance of *E. coli* isolates to  $\beta$ -lactams and fluoroquinolones. They found the presence of multidrug-resistant *E. coli* (66.8% of isolates). This is an alarming fact that indicates the importance of rationalizing the consumption of antibiotics. Pets are presented as a reservoir of multidrug resistance genes. A study in India, twenty *E. coli* isolates originating from the purulent uterus of dogs were found to be 100% resistant to tetracyclines, oxytetracyclines and gentamicin, and enrofloxacin (75%), ciprofloxacin (65%), amoxicillin (55%) (Bassessar et al. 2013). Boireau et al. (2018) examined *E. coli* isolates derived from animals used for human consumption, namely cattle, poultry and pigs. The research was conducted in the period from 2002 to 2015 in France. The highest antibiotic resistance of the

tested *E. coli* isolates was recorded for ceftiofur (about 22%), fluoroquinolones (30%), tetracyclines and amoxicillin (up to 90%), which is very high resistance and dangerous because these antibiotics are widely used and are very significant in veterinary and human medicine.

Resistance in poultry was (84%) in 2009 and decreased in 2015, especially on tetracyclines. In EU countries, the resistance of *E. coli* to antibiotics derived from poultry varies from 10% to over 70% (EFSA & ECDC 2018). Given that China is the largest consumer of antibiotics in the world, it is important to present certain studies in China that provide data on antibiotic resistance of *E. coli* isolates of certain animals (Zhang et al. 2015). In China, antibiotic resistance isolates of *E. coli* originating from chickens, ducks, pigs and cows have been tested. The research was conducted in the period from 2004 to 2012. The highest antibiotic resistance of all *E. coli* isolates was observed for tetracyclines, nalidixic acid, sulfamethoxazole, trimethoprim / sulfamethoxazole and ampicillin, and increasing resistance was also recorded for amikacin, aztreonam, ceftazidime, cefotaxime, chloramphenicol, ciprofloxacin. The spread of antibiotic resistance to reserve antibiotics is a great danger in the world. Multidrug-resistant *E. coli* from ducks (100%), chickens (82.2%), pigs (82.3%), cows (21.3%) was also isolated (Yassin et al. 2017). The data are alarming and require serious measures and control of the rational use of antibiotics. Monitoring data are important because they detect the risk of antibiotic resistance to take appropriate measures for the sustainability of effective antimicrobial therapy. Bosnia and Herzegovina still lacks adequate control measures to combat antibiotic resistance. At the *World Economic Forum* held in 2013, antibiotic resistance was presented as one of the major global risks to humans and animals (Schrijver et al. 2018).

## ANTIBIOTIC RESISTANCE OF *ESCHERICHIA COLI*

In human and animal isolates of *E. coli*, an increasing number of resistance genes have been identified in the last decade, acquired by horizontal gene transfer (Boireau et al. 2017; Poirel et al. 2018). This bacterial species acts both as a donor and as a recipient and can acquire resistance genes from other bacteria, but also transmit them to other bacteria (Poirel et al. 2018). The mechanism of resistance, enzymatic modification is considered to be the most common mechanism of acquired resistance of *E. coli*. A major discovery is considered to be the invention of irreversible  $\beta$ -lactamase inhibitors, such as clavulanic acid or sulbactam. In *E. coli*, several  $\beta$ -lactam resistance genes have been found. Some of them, such

as blaTEM-1 are widespread in *E. coli* of animal and human origin but encode only narrow-spectrum  $\beta$ -lactamases, which can inactivate penicillins and aminopenicillins (Poirel et al. 2018). A high level of resistance to penicillins and trimethoprim have been present for a long time, but it was low on the third generation of cephalosporins. Recently, however, an increase in resistance to reserve antibiotics (AB), including fluoroquinolones, has also been evident (Hooper & Jacoby 2016). Multidrug-resistant (MDR) isolates also occur in a healthy population due to the high potency of *E. coli*, which is caused by the uncontrolled use of AB in veterinary medicine, medicine, industry (Jelesić et al. 2011). The greatest danger is the possibility of the emergence of resistance on a global scale, to almost all cephalosporins, as well as the reserve antibiotic carbapenem (Papp-Wallace et al. 2015). More recently, in *E. coli*, derived from animals and humans, AmpCs genes have also been found, which also encode extended-spectrum  $\beta$ -lactamases (Poirel et al. 2018). Inactivation of III and IV generation of cephalosporins and carbapenems is associated with the production of extended-spectrum  $\beta$ -lactamases (Abraham et al. 2014). The mechanism of *E. coli* resistance may also be based on a decrease in bacterial membrane permeability to AB, bacterial enzymes, or an active efflux mechanism, which is characteristic of the emergence of antimicrobial resistance (AMR) to tetracycline AB. Resistance to these AB is more difficult and can be used for a longer period (Chetri et al. 2019). Bacteria quickly become resistant to aminoglycosides. By irrational use of these antibiotics, bacteria in a short time acquire the ability to produce several enzymes aminoglycoside acetyltransferase, nucleotidyltransferase and phosphotransferase, which inactivate this type of AB (Ramirez & Tolmasky 2010; Doi et al. 2016). Genes encoding aminoglycoside acetyltransferase enzymes have been found in acquired resistance to aminoglycoside AB, such as gentamicin (Tada et al. 2013). Resistance to sulfonamides occurs through a gradual chromosomal mutation, leading to an increase in the production of amino benzoic acid pairs, which results in a decrease in bacterial permeability to the same (Van Duijkeren et al. 2018). Trimethoprim resistance is based on the production of the enzyme dihydrofolate reductase encoded by genes (Capasso & Supuran 2014). Antimicrobial resistance can be caused by various biochemical mechanisms such as the mechanism of DNA gyrase and topoisomerase IV, decreased cell membrane permeability (porins), change of target site and active pump system, ejection of the drug from the bacterial cell (Poirel et al. 2018). Resistance of *E. coli* to fluoroquinolones and  $\beta$ -lactam carbapenems has already been reported (Tadesse et al. 2017). The evolution of resistance of *E. coli* differs

depending on the host, the mechanism of resistance, which are related to the mechanisms of action of AB to which the bacterium has acquired resistance (Boireau et al. 2017). In *E. coli*, the most common mechanism of resistance is enzymatic inactivation and modification (Poirel et al. 2018). There is also multidrug-resistant *E. coli*. These bacteria are resistant to more than three AB classes, but resistance to all AB is also often present (Qekwana et al. 2017; Hrustemović et al. 2021).

## CONCLUSION

*Escherichia coli* is a pluripotent bacterium, often multidrug-resistant, which indicates the importance of monitoring antibiotic resistance and establishing control over the use of antibiotics in veterinary medicine, especially in animals used for human consumption. Resistance genes can be transmitted from animal bacteria and food bacteria to human bacteria. The use of antibiotics in animals used for human consumption makes it possible to transfer resistance genes from food bacteria because food is never sterile to bacteria from humans. Antibiotics in animals raised for human consumption are often used to improve growth and to keep the food free of large amounts of bacteria. The application of the HACCP system in primary animal feed production reduces the population of food pathogens.

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