

Antimicrobial Resistance Profiling of Selected *E. coli* Isolates and Detection of ESBL/pAmpC-encoding Genes in Broiler Flocks in Bosnia and Herzegovina Using Real-Time PCR

Key words

antimicrobial resistance;
E. coli;
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Abstract: Antimicrobial resistance (AMR) is a growing global issue, driven by the nontargeted use of antimicrobials in livestock. Poultry, particularly broilers, may serve as significant reservoirs for resistant *Escherichia (E.) coli* strains. This study aimed to isolate *E. coli* from broiler flocks and evaluate their *in vitro* susceptibility towards β -lactams, cephalosporins, carbapenems, tetracyclines, and fluoroquinolones. Additionally, a multiplex real-time PCR assay was used to detect extended-spectrum β -lactamase (ESBL)- and carbapenemase-encoding genes. A total of 48 commensal *E. coli* isolates from broiler flocks in Bosnia and Herzegovina (BiH) were analyzed. Phenotypic resistance, determined using the disc diffusion method, was observed for ampicillin (87.5%), amoxicillin/clavulanic acid (62.5%), cefepime (41.7%), cefoxitin (45.8%), cefotaxime (50.0%), ceftazidime (47.9%), azithromycin (58.3%), ciprofloxacin (66.7%), and tetracycline (72.9%). PCR analysis confirmed *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{CMY} genes in 24 isolates (50%), whereas *bla*_{SHV} and carbapenemase-encoding genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48}, *bla*_{VIM} and *bla*_{GES}) were not detected. The high prevalence of multidrug-resistant *E. coli* strains highlights the need for enhanced antimicrobial stewardship in poultry production. Reducing antibiotic use, promoting alternative disease control measures, and implementing systematic resistance monitoring programs are crucial to reduce AMR in broiler farms and potential spill over to public health.

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Introduction

E. coli holds a unique position in microbiology, functioning as both a commensal bacterium and a potential pathogen (1). Most *E. coli* strains are non-pathogenic in mammals and poultry, constituting part of the normal intestinal flora (2). However, pathogenic strains can affect different organs and systems in diseased poultry, leading to conditions such as omphalitis, coligranulomatosis, air sac disease, avian cellulitis, swollen head syndrome, peritonitis, salpingitis, osteomyelitis, and panophthalmitis (2). Unlike in mammals,

where *E. coli* may cause intestinal diseases, in poultry, it primarily leads to secondary localized or systemic infections due to a weakened or insufficiently functional immune system (2). Additionally, *E. coli* infections can contribute to significant economic losses in the poultry industry (2). Young birds, typically between 2 and 12 weeks old, are most susceptible, with the highest mortality rates occurring around 4 to 9 weeks of age (3). The earliest indications of illness include reduced appetite, lethargy, and a dejected posture

with ruffled feathers. Infected birds may retract their heads and necks into their bodies, and as the condition progresses, they exhibit labored, rapid breathing, gasping, or other signs of respiratory distress (3).

Colibacillosis is a globally prevalent bacterial infection in poultry, with its incidence varying by region due to differences in management, climate, and biosecurity practices (4). It affects broilers, layers, breeders, and other species such as pigs, rabbits, and pet animals (4). Seasonal patterns influence its occurrence, with cold and wet conditions promoting *E. coli* survival and increasing disease outbreaks (4). Within poultry farms, factors like farm size, biosecurity, and stocking density play a crucial role in disease dynamics, with largescale farms being more vulnerable to outbreaks (4).

A significant concern associated with *E. coli* is the potential transmission through numerous pathways of virulent and/or antimicrobial-resistant strains, as these bacteria contribute to the global challenge of antimicrobial resistance and the transfer of resistance genes (1).

Antimicrobial resistance continues to pose a major threat to human and veterinary medicine (5). Although many AMR genes originate from the environment and resistance is a natural phenomenon, the intensive use of antibiotics in recent decades has been one of the main causes of the emergence and rapid spread of AMR (6, 7, 8). Another challenge in combating the spread of AMR is that it is evolving faster than the development and market introduction of new antimicrobial drugs (9). The use of antibiotics is widespread in commercial poultry production, one of the most widespread branches of animal production with an annual global production of over 90 billion tons of chicken meat in 2018 (10). An important milestone in the fight against the spread of AMR was the Regulation (EC) No 1831/2003 of the European Commission, which introduced an EU-wide ban on the use of antibiotics as growth promoters in the EU (11). In the United States of America (USA), similar legislation came into force in 2017 (12). Despite these efforts, the occurrence of resistant bacteria and antibiotic residues in poultry and poultry products remains a cause for concern (10). The digestive tract of poultry and other animals can serve as a reservoir for resistant bacteria such as *E. coli*, *Salmonella* and *Campylobacter* (13).

In Bosnia and Herzegovina, the "Law on Medicinal Products Used in Veterinary Medicine" (14) regulates the production, trade, testing and control of medicinal products used in veterinary medicine to protect animal health. It also defines the manner of prescribing and dispensing medicinal products and the conditions for trade of veterinary medicinal products (15, 16). However, the current regulations in BiH does not specify how and under what conditions antibiotics may be used in animal husbandry.

Of concern is that commensal *E. coli* strains can contribute to the spread of certain beta-(β)lactamases encoding genes known as extended spectrum β -lactamases (ESBL) encoding genes (13), as well as plasmid-mediated β -lactamases (pAmpC) (1). These enzymes are able to hydrolyze penicillins and cephalosporins up to the 3rd generation. ESBLs are often inhibited by clavulanic acid (18).

Poultry can be a potential source of ESBL-producing bacteria that can be transmitted to humans and lead to infections (19). Several EU Member States reported a high prevalence of ESBL-producing enterobacteria in European broiler production (20, 21, 22, 23, 24). In a study by Blaak et al. (23), ESBL-producing *E. coli* isolates were found to be highly prevalent in Dutch poultry production, especially in broiler flocks (81%), which could be related to AMU in broilers, which are more common than in layers. On the other hand, Mo et al. (22) state that the first ESBL-producing *E. coli* strain was isolated from the feces of a healthy broiler in 2006 and that broiler production in Norway could be a reservoir for ESBL-producing *E. coli* (24). Furthermore, Gao et al. (25) point out that ESBL-carrying *E. coli* can be spread from animals via the food chain and water. The ESBL-producing *E. coli* isolates were detected in the meconium of 1-day-old chicks, indicating that ESBL-encoding genes can be transmitted vertically (26).

In addition to ESBL-producing bacteria in livestock, carbapenem resistance in enterobacteria is also of increasing concern (27). In recent years, several studies have detected bacteria with carbapenemase-encoding genes in livestock and wild birds (28, 29, 30, 31, 32, 33, 34). The concern is justified by the importance of carbapenems as important antimicrobial agents (35) and their use in severe life-threatening infections with multidrug-resistant bacteria in humans (36, 37, 38). Due to their importance in human therapy, carbapenems are not authorized for use in food-producing animals in the EU (29).

Considering the sparse data on AMR in commensal *E. coli* isolates from commercial broiler production in BiH, the aim of this study was to isolate and identify *E. coli* from specific broiler farms and test their in vitro susceptibility to specific antibiotics from the group of β lactams, cephalosporins, carbapenems, tetracyclines and fluoroquinolones. In addition, a screening with multiplex real-time PCR was performed to identify the potential presence of potential ESBL- and carbapenemase-encoding genes.

Materials and methods

Sampling of diagnostic material was conducted between March 2019 and October 2020 on 48 broiler farms in BiH. The selection of these 48 flocks was based on a stratified random sampling method, ensuring representation from various regions within the country and accounting for factors such as farm size, production system, and biosecurity

practices. This approach aimed to capture a diverse range of conditions and practices that could influence the prevalence of *E. coli* infections. Broiler flocks consisted of at least 10 000 animals per farm. The sampled broiler flocks, all of COBB 500 provenance, were between 27 and 35 days old. Sampling was performed using two pairs of boot swabs per flock in the production area of each farm. The shoe covers were required to have adequate moisture absorption capacity and were pre-soaked with sterile 0.8% saline solution 0.8% saline (Centrohém d.o.o., Serbia). To ensure representative sampling, the designated area was walked through whole area. Upon completion, the boot swabs were carefully removed, turned inside out to retain the collected material, and properly packaged and labeled. Samples were transported to the laboratory as soon as possible in airtight, sealed containers under cold chain conditions. The time frame between sample collection and laboratory processing did not exceed 24 hours, ensuring sample integrity and minimizing potential changes in microbial composition.

Isolation of *E. coli*

The boot swabs were incubated for 18 ± 2 hours at 37 °C in 225 ml buffered peptone water (Condalab, Madrid, Spain). After incubation, the contents were inoculated onto MacConkey agar (Condalab, Madrid, Spain) to isolate *E. coli*, meanwhile another loopful was streaked onto MacConkey agar containing 1 mg/l cefotaxime (MC+CTX) (Acros Organics, China) to screen for *E. coli* resistant to third generation cephalosporins and incubated overnight at 37 °C. A presumptive *E. coli* colony was inoculated onto eosin methylene blue agar (Liofilchem s.r.l., Italy) and incubated overnight at 37 °C. API 20 E biochemical strips (BioMerieux, France) were used to confirm *E. coli* according to the manufacturer's instructions. Confirmed *E. coli* isolates were stored as stab cultures in nutrient agar for further antibiotic susceptibility testing and real-time PCR.

Antibiotic susceptibility testing (AST)

To test the antibiotic susceptibility of *E. coli* isolates, the disc diffusion method was performed according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) and the European Commission for Antimicrobial Susceptibility Testing (EUCAST) (39, 40). In addition, the Combination Disc Test (CDT) was performed to detect ESBL-producing *E. coli* isolates.

a) Disc diffusion method

For the disc diffusion method, isolates were suspended in sterile 0.8% saline solution and adjusted to a turbidity of 0.5 McFarland (BioMerieux, France) and inoculated uniformly onto Mueller-Hinton agar (Condalab, Madrid, Spain). After drying of the plate surface, antibiotic discs [ampicillin (10 µg), amoxicillin + clavulanic acid (20 µg + 10 µg), cefepime (30 µg), ceftazidime (30 µg), ceftazidime-clavulanic acid (30 µg + 10 µg), meropenem (10 µg), imipenem (10 µg), ertapenem

(10 µg), ciprofloxacin (5 µg), azithromycin (15 µg), tetracycline (30 µg), tigecycline (15 µg)] (Liofilchem s.r.l., Italy) were added and the plates were incubated overnight at 37 °C (39). Growth inhibition around the discs was measured the next day. The results were interpreted in accordance with the CLSI recommendations (39), with the exception of tigecycline, for which the interpretation was according to EUCAST (40). Depending on the diameter of the zone of inhibition, the *E. coli* isolates tested were classified as sensitive (S), intermediate (I), resistant (R) and susceptibility dosedependent (SDD). *E. coli* isolates were classified as SDD if the zone diameter for cefepime was 19-24 mm, as CLSI (39) does not have a breakpoint value for classifying isolates as "intermediate" for the above antibiotic. The interpretation categories and zone diameter breakpoints are listed in Table 1

Table 1; Interpretative categories and zone diameter in mm 600 breakpoints for selected antibiotics

Antibiotics	Interpretative categories and zone diameter breakpoints			
	S	I	R	SDD
Ampicillin	≥17	14-16	≤13	-
Amoxicillin + clavulanic acid	≥18	14-17	≤13	-
Cefepime	≥25	-	≤18	19-24
Cefoxitin	≥18	15-17	≤14	-
Cefotaxime	≥26	23-25	≤22	-
Ceftazidime	≥21	18-20	≤17	-
Meropenem	≥23	20-22	≤19	-
Ertapenem	≥22	19-21	≤18	-
Imipenem	≥23	20-22	≤19	-
Ciprofloxacin	≥26	22-25	≤21	-
Azithromycin	≥13	-	≤12	-
Tetracycline	≥15	12-14	≤11	-

b) Combination Disc Test (CDT)

E. coli isolates grown on MacConkey with 1 mg/l cefotaxime (Acros Organics, China) were subjected to CDT. For CDT, the *E. coli* isolates were adjusted to a turbidity of 0.5 McFarland (BioMerieux, France) and inoculated onto Mueller-Hinton agar (Condalab, Madrid, Spain). After drying the plate surface for 15 min, cefotaxime (30 µg), ceftazidime-clavulanic acid (30 µg and 10 µg), ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 plus 10 µg) (Liofilchem

s.r.l., Italy) were applied at least 25 mm apart from another (39). The test was positive if the zone of inhibition with clavulanic acid was ≥ 5 mm compared to that without clavulanic acid (e.g. ceftazidime = 16, ceftazidime + clavulanic acid = 21) (39). The *E. coli* strain ATCC 25922 was used as quality control.

Detection of potential AMR-associated genes

a) Bacterial DNA extraction by thermal lysis

The DNA template was prepared by the adapted boiling method according to Roschanski et al. (44). Thermal lysis was performed with 1 ml of an overnight culture of *E. coli* in BPW (Condalab, Madrid, Spain). The culture was centrifuged at 14 000 rpm for five minutes, the supernatant was discarded and the remaining pellet was resuspended in 300 μ l of sterile TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8). To lyse the cells, this was heated to 99 °C for 10 minutes. The suspension was cooled on ice for three minutes, vortexed centrifuged at 14000 rpm for two minutes. Finally, 100 μ l of the supernatant was stored at -20 °C for subsequent real-time PCR (44).

b) Real-time PCR for potential ESBL and carbapenemase-encoding AMR genes

The bacterial DNA of the isolates was subjected to a screening method by multiplex realtime PCR for the detection of AMR genes, using the specific primer pairs and fluorescent probes from previous studies (41, 42, 43). PCR cycling conditions for the detection of ESBL and carbapenemase-encoding genes were performed according to the protocol of Roschanski et al. (44) and van der Zee et al. (43).

Results

Isolation and identification of *E. coli*

Of a total of 48 broiler flocks examined, *E. coli* was detected in booth swabs from all broiler farms (n=48). All 48 isolates showed pink colour, shiny and smooth colony on the surface of MacConkey agar and characteristic metallic green sheen on EMB agar. By using CDT for ESBL identification, the presence of both ESBL-producing and non-producing *E. coli* isolates were confirmed. The results showed that 10 (20.83%) isolates were ESBL producers.

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility testing revealed variations in the susceptibility of *E. coli* isolates, as presented in Table 2. *E. coli* isolates derived from broiler farms showed the highest resistance rates, particularly to tetracycline (75%), ampicillin (72.92%), ceftazidime (66.67%), ciprofloxacin (31.25%), cefotaxime (20.83%), amoxicillin-clavulanic acid (18.75%), cefepime (14.58%),

Table 2: Results of antibiotic susceptibility testing using the disc diffusion method

Antibiotics	Interpretative categories and zone diameter breakpoints			
	S	I	R	SDD
Ampicillin	3 (6.25%)	10 (20.83%)	35 (72.92%)	-
Amoxicillin/ clavulanic acid	38 (79.17%)	1 (2.08%)	9 (18.75%)	-
Cefepime	22 (45.83%)	0%	7 (14.58%)	19 (39.58%)
Cefoxitin	46 (95.83%)	1 (2.08%)	1 (2.08%)	-
Cefotaxime	38 (79.17%)	0%	10 (20.83%)	-
Ceftazidime	14 (29.17%)	2 (4.16%)	32 (66.67%)	-
Meropenem	48 (100%)	0%	0%	-
Ertapenem	48 (100%)	0%	0%	-
Imipenem	48 (100%)	0%	0%	-
Ciprofloxacin	7 (14.58%)	26 (54.17%)	15 (31.25%)	-
Azithromycin	46 (95.83%)	0%	2 (4.17%)	-
Tetracycline	12 (25%)	0%	36 (75%)	-
Tigecycline	48 (100%)	0%	0%	-

azithromycin (4.17%), and cefoxitin (2.08%). Notably, the resistance was towards tigecycline or carbapenems (meropenem, ertapenem, and imipenem) was not detected.

Screening of potential ESBL-associated and carbapenemase-encoding AMR genes

Among the 48 *E. coli* isolates obtained from broiler farms, resistance genes encoding ESBL/pAmpC were identified—either individually or in combination—in 24 (50%) isolates as shown in Figure 1. Among these, the most prevalent resistance genes were *bla*_{TEM} (35.42%), *bla*_{CTX-M} (6.25%), and *bla*_{CMY} (2.08%). Some isolates carried combinations of resistance genes, including *bla*_{TEM+blaCMY} (4.17%) and *bla*_{TEM+blaCTX-M} (2.08%). Neither *bla*_{SHV} nor carbapenemase-encoding genes (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48}, *bla*_{VIM} and *bla*_{GES}) were detected.

Discussion

Antimicrobial resistance has become a major threat to public health, leading to problems in the prevention and treatment of persistent infections (44). Despite scientific efforts

Detected ESBL/pAmpC resistance genes

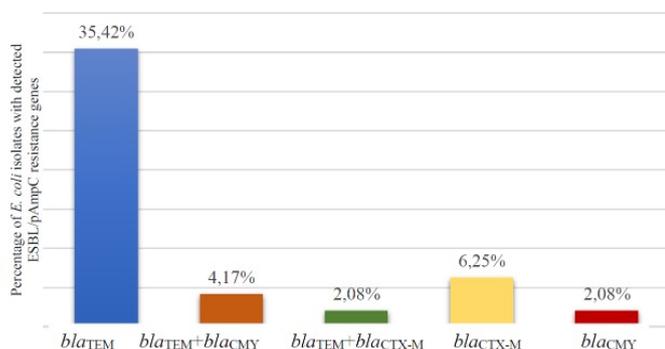


Figure 1: Detected ESBL/pAMPC resistance genes

to address this problem in recent decades, the spread of antimicrobial resistance remains a problem (44). The main reason for the emergence of AMR is considered to be AMU in veterinary, human medicine and in agricultural production (45). Antimicrobial resistance is influenced by spontaneous evolution, bacterial mutation and the transfer of resistant genes by horizontal gene transfer (44). It is particularly important to emphasize the role of Gram-negative bacteria such as *E. coli*, which are ubiquitous in humans and animals (1). *E. coli* can serve as a reservoir for AMR-encoding genes that can be transferred between different bacterial species, including zoonotic bacteria (1, 46, 47).

Among the *E. coli* isolates examined in this study, the highest resistance rate was found for tetracycline (75%), ampicillin (72.92%) and ceftazidime (66.67%), followed by ciprofloxacin (31.25%), cefotaxime (20.83%), amoxicillin + clavulanic acid (18.75%), cefepime (14.58%), azithromycin (4.17%) and ceftiofuran (2.08%) (Table 2). A similar observation was made by Persoons et al. (51), where high resistance to ampicillin, nalidixic acid, streptomycin, tetracycline and a combination of trimethoprim and sulfonamides was found in *E. coli* isolates from cloacal swabs and carcasses of broilers. Furthermore, the high levels of resistance to tetracyclines and ampicillin may be associated with the frequent use of these antibiotics in poultry production in our country. On the other hand, Much et al. (52) reported a high resistance rate to ciprofloxacin (69.1%) in *E. coli* isolated from appendix samples of conventionally reared broilers in Austria. In addition, Gholami-Ahangaran et al (50) reported a high rate of resistance to β -lactam antibiotics, macrolides, tetracyclines, fluoroquinolones, nitrofurans, aminoglycosides, folate pathway antagonists and phenicols in ESBL-producing *E. coli* isolates from turkey meat samples. According to these findings, it is hypothesized that the high resistance to β -lactams and other antibiotic classes may be due to the co-transfer of a plasmid carrying MDR-encoding genes (50).

In our study, 10 isolates showed resistance to cefotaxime. In a study by Hadžić-Hasanović et al (51), resistance to penicillins, nalidixic acid, cephalosporins and ceftiofuran was

found in *E. coli* isolates from chicken skin samples, including fresh and frozen meat samples, while Fetahagić et al. (52) reported resistance to amoxicillin, cefazolin, cefotaxime and ceftiofuran in cefotaxime-resistant *E. coli* isolates from poultry in BiH.

In addition, Gholami-Ahangaran et al. (50) state that AMR could be influenced by geographical location. Even though the resistance rate of *E. coli* varies in different European countries such as Poland, Germany, France and the United Kingdom, the resistance rate to penicillins is high in all countries (70%) (47). In addition, Blaak et al. (23) pointed out that ESBL-producing *E. coli* are widespread in poultry production in the Netherlands, especially in broiler flocks.

In the studies by Hadžić-Hasanović et al. (51) and Fetahagić et al. (52), phenotypically confirmed ESBL-producing *E. coli* isolates were sensitive to carbapenems. This was also confirmed in our study, as selected *E. coli* isolates were sensitive to meropenem, ertapenem and imipenem. According to the EFSA report 2021/2022 (53), resistance of *E. coli* to carbapenems was less common. In this regard, resistance to meropenem was detected in one isolate from broilers in 2021/2022. The less resistance to selected carbapenems can be explained by the fact that these antibiotics are not approved for use in food-producing animals (54, 55). According to Silva et al (56), the detection of carbapenemases in enterobacteria is less pronounced in *E. coli* and *Salmonella* spp. than in *Klebsiella (K.) pneumoniae*. This is confirmed by several studies from Europe in which sporadic carbapenem resistance was found in *E. coli* from pigs and their environment (33, 57, 58, 59). Resistance to tigecycline was found only in three isolates from broilers and pigs from Belgium and three isolates from pigs from Malta (53). No resistance to tigecycline was detected in our study.

Since β -lactams are used in veterinary and human medicine (60), the most common β -lactamases in Gram-negative bacteria contain genes encoding resistance to these antibiotics, represented by different genes belonging to *bla*_{TEM}, *bla*_{SHV}, *bla*_{OXA}, *bla*_{CMY} and *bla*_{CTX-M} (60, 61). In our study, the *bla*_{TEM} gene was the most represented (41.6%) (n=17) (Figure 1), but further molecular subtyping of this gene was not performed, so it is not possible to specify which subtype of *bla*_{TEM} it is and whether or not it is associated with ESBL production. A similar observation about the presence of the *bla*_{TEM} gene in *E. coli* isolates from poultry was made in a study from Thailand (65). The *bla*_{CTX-M} gene was found in three isolates that were also phenotypically resistant to cefotaxime. Gholami-Ahangaran et al (50) state that the presence of high resistance as well as the frequency of the *bla*_{CTX-M} gene can be associated with the presence of the ESBL gene and resistance to cefotaxime. It is important to point out that the combination of *bla*_{TEM/blaCMY} (n=2) and *bla*_{TEM/blaCTX-M} (n=1) genes was detected in some isolates. The *bla*_{CMY} gene was detected in one *E. coli* isolate. Previous studies have observed that combinations of different AMR genes are present in *E. coli* isolates (50, 63). Jafari et al.

(66) detected a combination of two or three ESBL genes in 15 (10 %) *E. coli* isolates from broiler chickens. The most frequent gene combinations in *E. coli* isolates were $bla_{TEM} + bla_{CTX-M-15}$, $bla_{TEM} + bla_{SHV}$, $bla_{SHV} + bla_{CTX-M-15}$ and as $bla_{TEM} + bla_{SHV} + bla_{CTX-M-15}$ type (66). In Iran, Gholami-Ahangaran et al (50) detected a combination of two ESBL genes in *E. coli* isolates from meat and intestinal contents of turkeys, with the most common gene combination being $bla_{TEM} + bla_{CTX-M}$. Molecular analysis in our study revealed that 7 out of 10 phenotypically confirmed ESBL-producing *E. coli* isolates carried at least one of the potential ESBL-associated genes: bla_{CMY} (n=1), bla_{CTX-M} (n=3), $bla_{TEM} + bla_{CMY}$ (n=2), and $bla_{TEM} + bla_{CTX-M}$ (n=1). On the other hand, in 3 out of 10 phenotypically confirmed ESBL-producing *E. coli* isolates, no AMR genes were detected using the molecular screening method. The lack of a genotype-phenotype association could be explained by other resistance genes (67) or factors that were not investigated in this study.

In addition, a larger panel of genes encoding β -lactamases could help determine the genetic determinants of resistance in these isolates (65). Furthermore, despite the presence of bla_{TEM} genes (n=17), no phenotypic resistance was observed. In this context, we did not characterize the subtypes of AMR genes detected, which would provide valuable information for a better understanding of the molecular AMR epidemiology. Discrepancies in genotype-phenotype association have been demonstrated in previous studies with clinical isolates of *E. coli* and *K. pneumoniae* (66, 67). Zhang et al. (66) investigated whether ESBL genes are present in clinical, non-ESBL-producing, antibiotic-susceptible *K. pneumoniae* isolates. A total of 8.9% (18/202) of the isolates carried only one ESBL gene, including the bla_{SHV} and bla_{CTX-M} genes. Nine of the isolates carrying ESBL genes did not exhibit phenotypic resistance (66). In their study, Kazemian et al. (67) observed the phenotypic and genotypic characteristics of ESBL-, AmpC- and carbapenemase-producing *K. pneumoniae* and *E. coli* recovered from clinical isolates. With the exception of one *E. coli* isolate, the genotypic method confirmed the phenotypic results (67). It is likely that the antibiotic regulates the antimicrobial resistance genes so that they are only slightly expressed *in vitro*, or that the phenomenon of heteroresistance associated with unstable tandem gene amplification, rare mutation and environmental modulation of the resistant genes may explain the different susceptibilities (64). Moreover, in a previous study, $bla_{CTX-M-1}$, bla_{TEM-52} and bla_{SHV-12} genes were observed as the most represented types of ESBL-encoding genes in poultry (26). An increase in the prevalence of the CTX-M type was previously described in the United Kingdom, the Netherlands and Germany (26). In the study by Hadžić-Hasanović (51), ESBL-producing *E. coli* isolates from BiH obtained from chicken skin contained different ESBL-encoding genes, including bla_{CTX-M} type genes, as well as mutations in TEM and SHV, encoding ESBL enzymes.

In different studies from African, American, Asian and European countries (43, 68, 59, 70, 71, 72, 73, 74, 75),

carbapenemase-encoding genes were detected in bacteria from farm animals (cattle, pigs, poultry), domestic animals (dogs, cats, ornamental birds) and wild animals. In Europe, carbapenemase-encoding genes are only sporadically detected in poultry. In our study, no carbapenemase-encoding resistance genes (bla_{KPC} , bla_{NDM} , bla_{OXA48} , bla_{VIM} and bla_{GES} genes) were detected. It should be noted that the route of transmission of carbapenemase-resistant enterobacteria from food-producing animals, pets and food is not yet fully understood (54). Although the origin of most carbapenemase-encoding genes is not fully understood, it is believed that carbapenemase-encoding genes are most likely acquired from environmental bacteria (76) or from the human field, where carbapenems are used to treat serious life-threatening human infections (77).

Recent studies highlight the ongoing challenges in managing antimicrobial resistance in veterinary pathogens. Gioushy et al. (78) emphasize the difficulty in treating *Mycoplasma bovis* mastitis due to variable resistance patterns, underlining the necessity of susceptibility-guided therapy. Given the increasing difficulties in treating resistant bacterial infections, alternative strategies are being explored. Phage therapy, in particular, is regaining attention. Stilec et al. (79) highlighted the potential of bacteriophages as precision tools that specifically target pathogenic bacteria, thus minimizing collateral damage to beneficial microbiota. Šumonja and Kotnik (80) further demonstrated the utility of phages in managing skin dysbiosis in atopic dogs, presenting a case for phage therapy as a viable alternative to antibiotics in veterinary medicine (80). These findings support the notion that alongside efforts to monitor antimicrobial gene spread, the veterinary field must begin integrating alternative treatment approaches.

To the best of our knowledge, this is among the few studies in Bosnia and Herzegovina that have identified ESBL and/or pAmpC resistance genes in *E. coli* isolates from broilers. Nevertheless, the study has certain limitations. The sample size was relatively small, and a more comprehensive investigation involving a larger number of samples and more detailed genetic characterization of the resistant isolates would have provided deeper insights. Antimicrobial resistance is a global challenge for veterinary and human medicine. Coordinated multidisciplinary initiatives focusing on antibiotic alternatives and reducing overall antibiotic consumption are being used to reduce antimicrobial resistance on chicken farms. It is necessary to develop and implement programs to monitor and control antibiotic resistance. In this regard, as in other countries, it is very important that the current legislation in BiH introduces a framework for the control of antibiotic use in the veterinary sector.

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Profiliranje protimikrobne odpornosti izbranih izolatov *E. coli* in odkrivanje genov, ki kodirajo ESBL/pAmpC, v jatah brojlerjev v Bosni in Hercegovini z uporabo PCR v realnem času

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Izveček: Protimikrobna odpornost (AMR) je vse večji globalni problem, ki ga povzroča neciljna uporaba protimikrobnih sredstev pri živini. Perutnina, zlasti brojlerji, lahko predstavlja pomemben rezervoar odpornih sevov *Escherichia (E.) coli*. Namen študije je bil izolirati *E. coli* iz jata brojlerjev in oceniti njihovo in vitro občutljivost za β -laktame, cefalosporine, karbapeneme, tetracikline in fluorokinolone. Poleg tega je bil za odkrivanje genov, ki kodirajo β -laktamaze z razširjenim spektrom (ESBL) in karbapenemaze, uporabljen hkratni PCR v realnem času. Analizirali smo skupno 48 izolatov komenzalne *E. coli* iz jata brojlerjev v Bosni in Hercegovini (BiH). Fenotipska odpornost, določena z metodo difuzije z diski, je bila opažena pri ampicilinu (87,5 %), amoksicilinu/klavulanski kislini (62,5 %), cefepimu (41,7 %), cefoksitinu (45,8 %), cefotaksimu (50,0 %), ceftazidimu (47,9 %), azitromicinu (58,3 %), ciprofloksacinu (66,7 %) in tetraciklinu (72,9 %). Analiza PCR je potrdila gene bla_{TEM} , bla_{CTX-M} in bla_{CMY} v 24 izolatih (50 %), medtem ko genov bla_{SHV} in genov, ki kodirajo karbapenemaze (bla_{KPC} , bla_{NDM} , bla_{OXA-48} , bla_{VIM} in bla_{GES}), nismo odkrili. Visoka prevalenca večkratno odpornih sevov *E. coli* poudarja potrebo po izboljšanjem upravljanju s protimikrobnimi sredstvi v perutninski proizvodnji. Zmanjšanje uporabe antibiotikov, spodbujanje alternativnih ukrepov za nadzor bolezni in izvajanje sistematičnih programov spremljanja odpornosti so ključni za zmanjšanje AMR na farmah brojlerjev in potencialnega prenosa na ljudi.

Ključne besede: protimikrobna odpornost; *E. coli*; brojlerji; Bosna in Hercegovina