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Research article

ANTIMICROBIAL RESISTANCE PROFILES OF *ESCHERICHIA COLI* ISOLATED FROM LAYING HENS REARED IN ORGANIC AND CONVENTIONAL HOUSING SYSTEMS

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Antimicrobial resistance (AMR) in *Escherichia coli* originating from poultry represents an important public health and veterinary concern. This study investigated phenotypic and genotypic AMR profiles of *E. coli* isolated from laying hens reared under different housing systems and assessed the occurrence of avian pathogenic *E. coli* (APEC). A total of 60 *E.coli* isolates were obtained from fecal samples collected from laying hens reared in organic, cage, and floor housing systems across multiple production sites operating under the same poultry production system in Serbia. Antimicrobial susceptibility was determined using the disk diffusion method and selected AMR and virulence-associated genes were detected by PCR. Regardless of the housing system, the highest levels of phenotypic resistance were observed for tetracycline, ampicillin, and nalidixic acid. Resistance to critically important antimicrobials, including fluoroquinolones, gentamicin, and meropenem, was detected exclusively or predominantly in isolates from floor housing systems. ESBL-producing *E. coli* and APEC strains were most frequently identified in floor-housed flocks, whereas isolates from the organic production system showed the lowest prevalence of ESBL producers and multidrug-resistant (MDR) strains. Genotypically, the highest proportion of MDR isolates was detected in the cage housing system. The *mcr-1* gene was identified in a single isolate originating from the cage system. These findings indicate that housing conditions may influence the distribution of AMR and virulence traits in *E. coli*. The study provides the first comparative data of this type from Serbia and highlights the importance of continued AMR surveillance across different poultry production systems.

Keywords: antibiotic resistance; cage housing systems; floor housing system; laying hens; organic farming; poultry

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INTRODUCTION

Escherichia coli is a Gram-negative, ubiquitous bacterium that forms a natural part of the intestinal microbiome of humans and animals. While most strains are harmless commensals, some possess the ability to cause disease in both humans and animals. Avian Pathogenic *E. coli* (APEC) represents a group of virulent *E. coli* strains responsible for colibacillosis in poultry, one of the most frequent bacterial diseases and a major cause of economic losses in intensive poultry production [1-3].

On a global level, the increasing prevalence of antimicrobial resistance (AMR) represents one of the major challenges to public health [4]. Owing to its genetic flexibility and capacity for rapid adaptation, *E. coli* has developed multiple resistance mechanisms and acts as an efficient donor, recipient, and reservoir of resistance genes [5,6]. For this reason, *E. coli* has been recommended as a key indicator organism for AMR surveillance within monitoring programs of the World Health Organization (WHO), the World Organisation for Animal Health (WOAH), and within the European Union regulatory framework for harmonized monitoring of antimicrobial resistance in zoonotic and commensal bacteria [7-9]. In addition to resistance to antimicrobials approved for veterinary use, *E. coli* isolates from poultry are increasingly found to exhibit resistance to antibiotics predominantly or exclusively used in human medicine [10]. An important resistance mechanism in *E. coli* is the production of extended-spectrum β-lactamases (ESBLs), which confer resistance to extended-spectrum cephalosporins and are frequently associated with multidrug-resistant (MDR) phenotypes. ESBL-producing *E. coli* are of particular concern due to their limited therapeutic options and their increasing occurrence in both human and animal populations [10,11]. Poultry represents an important reservoir of MDR-ESBL-producing *E. coli*, which can be transmitted to humans through direct contact with animals, contaminated food, or exposure to contaminated environments [11,12].

Global population growth has led to an increased demand for food, including poultry meat and eggs [13]. At the same time, growing awareness of health and environmental concerns associated with intensive livestock production and antimicrobial use has contributed to changes in animal production systems. As a result, alternative farming practices, such as organic poultry production, have gained increasing attention, as these systems restrict or exclude the use of antibiotics and other synthetic inputs [8,10,14-16]. However, data comparing antimicrobial resistance patterns in *E. coli* from conventional and organic poultry production systems remain limited and, in some cases, inconsistent, particularly with regard to specific resistance genes [12].

The aim of this study was to investigate AMR in *E. coli* isolated from the feces of laying hens and to compare resistance patterns and the occurrence of APEC strains between conventional (cage and floor) and organic production systems, in order to assess whether housing conditions potentially influence the prevalence of specific AMR genes.

MATERIAL AND METHODS

Ethics and informed consent

This study did not involve any experimental procedures or interventions on animals. Fecal samples were collected non-invasively during routine farm management, without causing pain, stress, or unnecessary suffering to the animals. The study protocol was reviewed and approved by the Ethics Committee of Faculty of Veterinary medicine, Belgrade, Serbia (Approval No. 02-02-2025).

Sample collection and bacterial isolation and identification of *E. coli*

Fecal samples were collected from laying hens of the Lohmann Brown hybrid breed, aged six months, housed in organic, cage, and floor housing systems at separate facilities within the same poultry production system in Serbia. All birds were in the production phase and had not been exposed to antibiotics during their lifetime. A total of 60 fecal samples were collected by random sampling, including 20 samples from an organic production system and 40 samples from conventional production systems, comprising 20 samples from floor housing and 20 from cage housing. Following aseptic sampling, each specimen was labeled and transported to the laboratory under refrigerated conditions.

Isolation of *E. coli* was performed on the same day by inoculating fecal samples on blood agar supplemented with 5% sheep blood (*Becton Dickinson, USA*) and MacConkey agar (*Becton Dickinson, USA*), followed by incubation at 37 °C for 24 h. After obtaining pure cultures, strain identification was carried out using conventional biochemical tests (indole, methyl red, Voges-Proskauer, and citrate tests) and subsequently confirmed using the API 20E identification system (*bioMérieux, France*). A total of 60 *E. coli* isolates were selected for further analyses, with 20 isolates originating from each housing system (cage, floor, and organic).

DNA extraction and identification of APEC strains

Genomic DNA was extracted using the GeneJET DNA Purification Kit (*Thermo Scientific, USA*) in accordance with the manufacturer's protocol for Gram-negative bacteria. The extracted DNA was stored at −20 °C until further analysis.

Identification of APEC strains was performed by multiplex PCR based on the detection of specific virulence-associated genes (*iroN, ompT, hlyF, iss,* and *iutA*), which are considered minimal predictors of the APEC pathotype [17]. PCR products were visualized by agarose gel electrophoresis (*Serva, Germany*). Each PCR run included a positive control consisting of DNA from a previously characterized APEC strain carrying the target virulence genes. Negative controls were included in each PCR reaction.

Antimicrobial susceptibility testing and phenotypic detection of ESBL production

Phenotypic antimicrobial susceptibility of *E. coli* isolates to selected groups of antibiotics was determined using the disk diffusion method on Mueller-Hinton agar (*Becton Dickinson, USA*), in accordance with the standards and interpretative criteria defined by the European Committee on Antimicrobial Susceptibility Testing [18]. The following antimicrobial agents were tested: ampicillin 10 μg (AMP), amoxicillin/clavulanic acid 20/10 μg (AMC), cefotaxime 30 μg (CTX), ceftazidime 30 μg (CAZ), meropenem 10 μg (MEM), nalidixic acid 30 μg (NAL), ciprofloxacin 5 μg (CIP), tetracycline 30 μg (TET), gentamicin 10 μg (GEN), trimethoprim 5 μg (TMP), chloramphenicol 30 μg (CHL), tigecycline 15 μg (TGC), and sulfamethoxazole/trimethoprim 1.25/23.75 μg (SXT) (*Becton Dickinson, USA*). Quality control of antimicrobial susceptibility testing was performed using the reference strain *E. coli* ATCC 25922, in accordance with EUCAST recommendations [18].

The ability of *E. coli* isolates to produce extended-spectrum β-lactamases (ESBLs) was assessed using the combined disk test recommended by the Clinical and Laboratory Standards Institute [19]. For this purpose, disks containing cefotaxime 30 μg (CTX), cefotaxime/clavulanic acid 30/10 μg (CTX/CLA), ceftazidime 30 μg (CAZ), and ceftazidime/clavulanic acid 30/10 μg (CAZ/CLA) were used (*Becton Dickinson, USA*). ESBL-positive and ESBL-negative control strains were included to validate the combined disk test.

Detection of antimicrobial resistance genes

The presence of AMR genes was determined using three different PCR protocols, including two multiplex PCR assays and one PCR assay for the detection of colistin resistance, as previously described [20-22]. PCR products were visualized by agarose gel electrophoresis (*Serva, Germany*). Each PCR assay included a positive control consisting of DNA from a previously characterized strain carrying the respective AMR gene, as well as a negative control with nuclease free water. The resistance genes analyzed in this study, along with the primers used, are listed in Table 1.

All *E. coli* strains carrying resistance genes for at least three different antibiotic classes were characterized as MDR strains, following the criteria established by Magiorakos et al. [23].

RESULTS

The results of the detection of virulence genes characteristic of APEC strains are presented in Table 2. Out of a total of 60 *E. coli* isolates, five isolates carried four of the five investigated virulence genes and were therefore classified as APEC strains. These included three isolates originating from the floor housing system and one isolate each from the organic and cage housing systems.

Table 1. Primers used for the detection of APEC strains and selected AMR genes

Gene	Primer sequences (5'-3')	Amplicon size (bp)	Description	Reference for primers and protocols
iroN	AATCCGGCAAAGAGACGAACCGCCT GTTCGGGCAACCCCTGCTTTGACTTT	553	Gene encoding the salmochelin siderophore receptor (APEC-associated virulence predictor)	[17]
ompT	TCATCCCGGAAGCCTCCCTCACTACTAT TAGCGTTTGCTGCACTGGCTTCTGATAC	496	Episomal gene encoding an outer membrane protease (APEC-associated virulence predictor)	[17]
hlyF	GGCCACAGTCGTTTAGGGTGCTTACC GGCGGTTTAGGCATTCCGATACTCAG	450	Putative avian hemolysin (APEC-associated virulence predictor)	[17]
iss	CAGCAACCCGAACCACTTGATG AGCATTGCCAGAGCGGCAGAA	323	Episomal gene associated with increased serum survival (APEC-associated virulence predictor)	[17]
iutA	GGCTGGACATCATGGGAACTGG CGTCGGGAACGGGTAGAATCG	302	Gene encoding the aerobactin siderophore receptor (APEC-associated virulence predictor)	[17]
qnrA	AGAGGATTTCTCACGCCAGG TGCCAGGCACAGATCTTGAC	580	Fluoroquinolone resistance	[21]
qnrB	GGMATHGAAATTCGCCACTG TTTGCYGYYCGCCAGTCGAA	264	Fluoroquinolone resistance	[21]
qnrS	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	Fluoroquinolone resistance	[21]
tetA	CGGGGCGACTGGGGCGGTAGC CAAAGCGCGGCCGGCACCTGT	372	Tetracycline resistance	[22]
tetB	AACGCGTGAAGTGGTTCGGTTGGT TTCGCCCCATTTAGTGGCTATTCTTC	446	Tetracycline resistance	[22]
blaTEM	ATGTGCGCGGAACCCCTATTTGTTTA AAAAAGCGGTTAGCTCCTTCGGTCCT	558	Resistance to ampicillin and amoxicillin	[22]
aph(3)IA	TCGGGCAATCAGGTGCGACAATCTA TGCCAGCGCATCAACAATATTTTCACC	278	Aminoglycoside resistance (neomycin, kanamycin)	[22]
aac3VI	GGCACCCGCGACGCCCTGGTCCAAAAG GGGCCCGGCGCCGATCGACAGGATTT	502	Aminoglycoside resistance (gentamicin)	[22]
mcr-1	CGGTCAGTCCGTTTGTTC CTTGGTCGGTCTGTAGGG	308	Colistin resistance	[20]

Table 2. Results of multiplex PCR analysis for virulence genes characteristic of APEC strains

Virulence gene	*IroN* n (%)	*ompT* n (%)	*Iss* n (%)	*hlyF* n (%)	*iutA* n (%)	APEC strain n (%)
Cage housing system *n*=20	0 (-)	0 (-)	2 (10%)	1 (5%)	1 (5%)	1 (5%)
Floor housing system *n*=20	1 (5%)	2 (10%)	2 (10%)	5 (25%)	10 (50%)	3 (15%)
Organic production system *n*=20	1 (5%)	3 (15%)	2 (10%)	0 (-)	1 (5%)	1 (5%)

The results of antimicrobial susceptibility testing of *E. coli* isolates determined by the disk diffusion method are presented in Table 3. In the organic production system, the proportion of resistant isolates was 55% to ampicillin and 60% to tetracycline, while in the floor housing system resistance rates were 50% to ampicillin and 45% to tetracycline. In the cage housing system, the proportion of isolates resistant to both antibiotics was 35%. Resistance to nalidixic acid was highest in the floor housing system, reaching 60%. Resistance to AMC, ceftazidime, trimethoprim, and SXT was detected at frequencies below 30% across all three production systems. Resistance to chloramphenicol was observed in isolates from the floor and organic housing systems, whereas all isolates from the cage housing system were susceptible to this antibiotic. Resistance to ciprofloxacin (25%), meropenem (15%), and gentamicin (5%) was detected exclusively in isolates originating from the floor housing system, while resistance to cefotaxime (15%) was observed only in isolates from the cage housing system. All isolates were susceptible to tigecycline.

The number of isolates positive for the ESBL test was highest in the floor housing system (35%) and lowest in the organic production system (10%). The results are presented in Table 4.

Table 5 presents the results of PCR-based AMR gene detection and the number of *E. coli* isolates classified as MDR based on the detected resistance genes. Among the investigated genes, the highest prevalence of resistance was observed for tetracyclines, with *tetA* detected in 45–55% of isolates and *tetB* in 5–20% of isolates. The highest proportion of MDR strains was observed in the cage housing system (35%), while the lowest proportion was recorded in the organic production system (5%).

Table 3. Results of antimicrobial susceptibility testing of *E. coli* determined by the disk diffusion method

Antibiotic	Cage housing system n=20			Floor housing system n=20			Organic production system n=20		
	S* n (%)	I** n (%)	R*** n (%)	S* n (%)	I** n (%)	R*** n (%)	S* n (%)	I** n (%)	R*** n (%)
AMP	13 (65%)	0 (-)	7 (35%)	10 (50%)	0 (-)	10 (50%)	8 (40%)	1 (5%)	11 (55%)
AMC	19 (95%)	0 (-)	1 (5%)	14 (70%)	1 (5%)	5 (25%)	18 (80%)	1 (5%)	1 (5%)
CTX	17 (85%)	0 (-)	3 (15%)	19 (95%)	1 (5%)	0 (-)	20 (100%)	0 (-)	0 (-)
CAZ	16 (80%)	0 (-)	4 (20%)	19 (95%)	0 (-)	1 (5%)	17 (85%)	0 (-)	3 (15%)
MEM	20 (100%)	0 (-)	0 (-)	17 (85%)	0 (-)	3 (15%)	20 (100%)	0 (-)	0 (-)
NAL	17 (85%)	1 (5%)	2 (10%)	8 (40%)	0 (-)	12 (60%)	17 (85%)	0 (-)	3 (15%)
CIP	20 (100%)	0 (-)	0 (-)	15 (75%)	0 (-)	5 (25%)	20 (100%)	0 (-)	0 (-)
TET	13 (65%)	0 (-)	7 (35%)	9 (45%)	2 (10%)	9 (45%)	8 (40%)	0 (-)	12 (60%)
GEN	20 (100%)	0 (-)	0 (-)	17 (85%)	2 (10%)	1 (5%)	20 (100%)	0 (-)	0 (-)
TMP	18 (90%)	0 (-)	2 (10%)	14 (70%)	0 (-)	6 (30%)	17 (85%)	0 (-)	3 (15%)
CHL	20 (100%)	0 (-)	0 (-)	18 (90%)	0 (-)	2 (10%)	19 (95%)	0 (-)	1 (5%)
TGC	20 (100%)	0 (-)	0 (-)	20 (100%)	0 (-)	0 (-)	20 (100%)	0 (-)	0 (-)
SXT	19 (95%)	0 (-)	1 (5%)	14 (70%)	0 (-)	6 (30%)	17 (85%)	0 (-)	3 (15%)

*susceptible, **intermediate, ***resistant

Table 4. Results of the combined disk test for phenotypic detection of ESBL-producing *E. coli*

	ESBL-positive isolates by housing system (n (%))
Cage housing system	5 (25%)
Floor housing system	7 (35%)
Organic production system	2 (10%)

Table 5. Results of PCR analysis of antibiotic resistance genes

Gene (antibiotic resistance)	Cage housing system n (%)	Floor housing system n (%)	Organic production system n (%)
blaTEM (ampicillin, amoxicillin)	5 (25%)	6 (30%)	1 (5%)
aac3VI (gentamicin)	5 (25%)	7 (35%)	7 (35%)
aph(3)IA (neomycin, kanamycin)	11 (55%)	0 (-)	2 (10%)
tetB (tetracyclines)	0 (-)	4 (20%)	1 (5%)
tetA (tetracyclines)	10 (50%)	9 (45%)	11 (55%)
qnrA (fluoroquinolones)	2 (10%)	2 (10%)	3 (15%)
qnrB (fluoroquinolones)	7 (35%)	1 (5%)	3 (15%)
qnrS (fluoroquinolones)	5 (25%)	10 (50%)	5 (25%)
mcr-1 *(colistin)*	1 (5%)	0 (-)	0 (-)
Number of MDR isolates n (%)	7 (35%)	3 (15%)	1 (5%)

DISCUSSION

Regardless of the housing system, the highest proportion of isolates exhibited phenotypic resistance to tetracycline, ampicillin, and nalidixic acid, which is consistent with their extensive historical use in poultry production [11]. Molecular analysis showed a predominance of the *tetA* gene over *tetB* across production systems, in line with previous reports identifying *tetA* as the dominant tetracycline resistance determinant in Gram-negative bacteria [24,25]. Although phenotypic resistance to ampicillin was highest among isolates from the organic production system, the *blaTEM* gene was more frequently detected in isolates from conventional housing systems. This discrepancy suggests that ampicillin resistance in organic systems may be mediated by alternative mechanisms, such as other β-lactamase genes, reduced membrane permeability, or efflux activity, which were not investigated in the present study [26]. The *blaTEM* gene is among the most widespread β-lactam resistance determinants in Enterobacterales worldwide [27] and has previously been reported as plasmid-located in the majority of *E. coli* isolates from Serbia [28]. Resistance to third-generation cephalosporins was low, which is consistent with the restricted use of these antimicrobials in poultry production. Phenotypic resistance to meropenem was detected only in three isolates from floor-housed laying hens, which is consistent

with the extremely rare occurrence of carbapenem resistance in poultry-associated *E. coli* in Europe [12]. Given that carbapenems are not authorized for use in food-producing animals [29], this finding likely reflects indirect mechanisms such as co-selection by other β-lactam antibiotics or combined effects of β-lactamase production and reduced membrane permeability, rather than direct selective pressure [30,31] . Resistance to fluoroquinolones is of particular concern due to their classification as High Priority Critically Important Antimicrobials (HPCIA) by the WHO [32]. The *qnrS* gene is among the most frequently reported and globally widespread plasmid-mediated fluoroquinolone resistance genes [33], which is consistent with our findings. Overall, both phenotypic resistance to quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin) and genotypic resistance to fluoroquinolones were most prevalent among isolates originating from floor housing systems. Resistance to chloramphenicol was detected at low frequency, despite its long-standing ban in food-producing animals [34]. This finding likely reflects co-selection driven by the use of other antimicrobials, including florfenicol, for which cross-resistance mediated by the *floR* gene has been described [34-36]. All isolates remained susceptible to tigecycline, which is consistent with reports of the rare occurrence of tigecycline resistance among poultry-derived *E. coli* in Europe [37]. Colistin represents a last-line treatment for infections caused by MDR Gram-negative bacteria; therefore, any detection of colistin resistance in *E. coli* is epidemiologically relevant [38]. In Serbia, colistin resistance in *E. coli* of veterinary origin was first reported in 2021 in isolates from turkeys [39]. In the present study, the *mcr-1* gene was identified in a single isolate from the cage housing system. Although *mcr-1* was originally described as a plasmid-mediated colistin resistance mechanism [40], its genetic location and transferability were not investigated in the present study. Furthermore, because colistin susceptibility was not determined using an MIC-based reference method, the phenotypic resistance of this isolate could not be established according to EUCAST criteria [41].

The detection of ESBL-producing *E. coli* is of concern, as poultry production is considered one of the main sources of ESBL-producing *E. coli* for humans [42]. Dominant *E. coli* lineages harboring ESBL determinants, especially *bla*CTX-M variants, have been identified in human and veterinary populations, indicating the continuity of AMR across the farm-to-fork chain [43,44]. These strains are frequently MDR and potentially pathogenic [42]. In line with these findings, our results show that four of the fourteen ESBL-producing *E. coli* isolates were classified as MDR based on the detected resistance genes, while three were identified as APEC strains. Notably, one ESBL-producing isolate was phenotypically resistant to seven of the tested antibiotics.

APEC is considered a potential reservoir of virulent and AMR *E. coli* strains with zoonotic potential, with the poultry intestinal tract representing the primary reservoir [1,45]. The occurrence of plasmids carrying both virulence and AMR genes in *E. coli* is therefore of particular concern [6]. In the present study, three out of five APEC isolates were classified as MDR based on the detected resistance genes, and all originated from floor housing systems.

When comparing housing systems, differences in the distribution of AMR patterns were observed.

Organic production system

In recent decades, increasing attention has been given to alternative livestock production systems aimed at improving animal health and reducing antibiotic use, including organic poultry production, as well as the application of probiotics, prebiotics, and autogenous vaccines [11]. Organic production systems are based on reduced use of pharmaceuticals and improved management practices, with the goal of enhancing animal welfare and decreasing the incidence of infectious diseases, thereby reducing the need for therapeutic antibiotic application [46,47]. Isolates originating from the organic production system showed the lowest prevalence of ESBL-producing *E. coli* and the lowest proportion of MDR isolates which is consistent with findings reported by other authors [10-12,16, 47]. Recent investigations in broilers indicate that alternative or non-conventional production systems are generally associated with a lower prevalence of resistant *E. coli* in caecal contents [42,47,48] and in neck skin samples collected at slaughter [44], compared with intensive production systems. In line with these findings, isolates from the organic production system in the present study showed limited phenotypic resistance, mainly to tetracycline, ampicillin, and nalidixic acid, while resistance to critically important antimicrobials was rare or absent and only one APEC isolate was detected.

The occurrence of AMR is multifactorial, and differences observed between organic and conventional production systems cannot be attributed to a single factor, but rather to a combination of management, environmental, and biological influences [49]. However, factors such as stocking density and feeding practices are likely to play an important role in limiting the emergence and spread of AMR. Reduced stocking density has been associated with a lower incidence of infectious diseases and, consequently, with a reduced need for therapeutic antibiotic use [7,46,47]. Based on farm records and on-site observations, laying hens in the organic production system included in this study were fed organically certified feed, in accordance with the stricter requirements defined by national legislation on organic production [50]. In addition, hens reared under organic conditions were kept at lower stocking densities and had access to outdoor areas, in contrast to conventional systems where birds are housed at higher densities with limited movement and no outdoor access. Close and prolonged contact between birds in conventional housing systems is considered to facilitate the transmission of infections and antimicrobial resistance genes, which may contribute to higher resistance levels. Furthermore, differences in gut microbiota composition associated with organic feeding practices may contribute to increased colonization resistance against pathogenic and resistant bacteria [51]. Also, lower stress levels associated with improved welfare conditions may also indirectly influence susceptibility to infection and bacterial shedding [52].

Nevertheless, a considerable proportion of resistance to certain antibiotics was also observed in the organic production system, as phenotypic resistance to tetracycline and ampicillin was higher in organic isolates compared with conventional systems. This indicates that the presence of AMR bacteria in organic poultry production cannot be attributed solely to on-farm antimicrobial use. Environmental sources, including wildlife, insects, wastewater, and manure, may contribute to environmental contamination and represent important reservoirs of antimicrobial resistance [10]. In a study conducted in Germany, approximately 5% of wild birds were found to carry multidrug-resistant *E. coli*, most frequently exhibiting resistance to ampicillin and tetracycline [53]. In addition, vertical transmission from parent flocks, contamination at hatcheries, during transport, or residual contamination of poultry houses may contribute to the occurrence of resistant and MDR *E. coli* in organically reared poultry [11,47]. It should also be noted that AMR can persist within bacterial populations over time, even in the absence of direct selective pressure, which may further explain the detection of resistant isolates under organic production conditions [54].

Conventional housing system

In this study, the cage housing system was characterized by moderate levels of phenotypic resistance and the highest proportion of MDR isolates, with frequent detection of AMR genes. One APEC isolate and a single *mcr-1*–positive isolate was detected in this system. Overall, these findings are in line with previous studies reporting higher levels of AMR in intensive poultry production systems [7,47] and resistance patterns described across different poultry production systems [12].

The floor housing system exhibited the highest overall levels of phenotypic and genotypic AMR. Isolates from this system showed the highest resistance to quinolones and fluoroquinolones, with increased resistance to several commonly used antimicrobials, while phenotypic resistance to ciprofloxacin, gentamicin, and meropenem was detected exclusively in floor-housed isolates. In addition, the highest prevalence of ESBL-producing *E. coli* and APEC strains was observed in this system, and all MDR-APEC isolates originated from floor-housed flocks. These findings are consistent with previous studies reporting elevated AMR in poultry production systems characterized by continuous and direct contact with the farm environment. In floor-based systems, birds remain in permanent contact with litter throughout the production cycle, which has been shown to accumulate AMR *E. coli* and facilitate repeated exposure and re-colonization of birds [7,47]. Although the persistence of resistant bacteria in the farm environment and indirect transmission via contaminated equipment and personnel occur across different poultry production systems, these processes may be more pronounced in floor-based systems due to prolonged environmental exposure and increased manual handling [7,47]. The higher prevalence of ESBL-producing *E. coli* and APEC strains observed in this system is in line with reports describing the frequent co-occurrence of virulence and resistance traits in poultry-associated *E. coli*, without implying direct causality [6,42].

Organic poultry production is characterized by a set of management practices that collectively create conditions associated with a lower prevalence of AMR, suggesting its potential role in mitigating the spread of AMR. However, further research is needed to identify and disentangle individual factors contributing to this effect and to clarify their relative impact on AMR. The knowledge gained from such studies should be used to support further optimization and harmonization of management practices, as well as the development of evidence-based guidelines for organic poultry production. Continuous AMR monitoring remains essential. This study has several limitations, including the relatively small sample size and sampling restricted to multiple production sites operating under the same poultry production system, which may limit the applicability of the finding to other poultry production systems. Molecular analysis was limited to selected AMR genes used for the classification of MDR isolates and inclusion of a broader panel of resistance determinants would likely result in a higher estimated prevalence of MDR. Futhermore, molecular characterization of the phenotypically confirmed ESBL-producing isolates was limited. Although *blaTEM* was included in the PCR panel, the assay did not characterize specific *blaTEM* variants, while other relevant ESBL- and AmpC-associated genes, including *blaCTX-M, blaSHV,* and *blaCMY-2*, were not investigated. Therefore, the genetic determinants responsible for the observed ESBL phenotypes could not be established. Future studies should include a broader molecular characterization of β-lactamase genes in phenotypically confirmed ESBL-producing isolates.

It should be noted that this represents the first study of its kind conducted in the Republic of Serbia, where formal guidelines for the rational use of antimicrobials in veterinary medicine remain limited.

CONCLUSIONS

Isolates from the organic system showed the lowest prevalence of phenotypically confirmed ESBL producers and genotypically defined MDR strains, whereas isolates from the floor system exhibited the highest overall phenotypic resistance and the highest occurrence of ESBL-producing and APEC isolates. The cage system had the highest proportion of genotypically defined MDR isolates and included the only *mcr-1*-positive isolate. These findings suggest that housing and management conditions may be associated with differences in the distribution of AMR in poultry-associated *E. coli* and support continued surveillance across different production systems.

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Authors' contributions

MMilinković, IP, AN and MMilanović carried out the primary bacteriology analysis including sample collection, culturing, bacteria and biochemical analysis. AR, KA, NZ and AN performed the molecular analysis. MMilinković, IP, AR, JN and DK conceived the study, participated in its design and coordination and revised the final version of the manuscript. MMilinković and IP came up with the draft of the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ANTIMIKROBNA REZISTENCIJA SOJEVA *ESCHERICHIA COLI* IZOLOVANIH OD KOKOŠAKA NOSILJA GAJENIH U ORGANSKIM I KONVENCIONALNIM SISTEMIMA UZGOJA

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Antimikrobna rezistencija (AMR) kod *Escherichia coli* poreklom od živine predstavlja problem za javno zdravlje i značajan problem u veterinarskoj medicini. U ovoj studiji ispitani su fenotipski i genotipski profili AMR kod sojeva *E. coli* izolovanih od kokošaka nosilja gajenih u različitim sistemima držanja, kao i učestalost pojave avijarne patogene *E. coli* (APEC). Ukupno je analizirano 60 izolata *E. coli* dobijenih iz fekalnih uzoraka kokošaka nosilja gajenih u organskom, kaveznom i podnom sistemu uzgoja na više proizvodnih lokacija koje posluju u okviru istog živinarskog proizvodnog sistema u Republici Srbiji. Osetljivost na antibiotike određena je metodom disk-difuzije, dok je prisustvo odabranih gena rezistencije i gena povezanih sa virulencijom utvrđeno PCR metodom. Nezavisno od sistema uzgoja, najviši nivoi fenotipske rezistencije zabeleženi su na tetraciklin, ampicilin i nalidiksinsku kiselinu. Rezistencija na kritično važne antimikrobne lekove, uključujući fluorohinolone, gentamicin i meropenem, zabeležena je isključivo ili pretežno kod izolata iz podnog sistema uzgoja. ESBL-produkujući sojevi *E. coli* i APEC sojevi najčešće su identifikovani u podnom sistemu, dok su izolati iz organskog sistema uzgoja pokazali najnižu prevalenciju ESBL sojeva i multirezistentnih (MDR) izolata. Na genotipskom nivou, najveći udeo MDR izolata zabeležen je u kaveznom sistemu uzgoja. Gen *mcr-1* detektovan je kod jednog izolata poreklom iz kaveznog sistema. Dobijeni rezultati ukazuju na to da uslovi držanja mogu uticati na AMR kod *E. coli*. Ovo istraživanje predstavlja prve komparativne podatke ove vrste u Republici Srbiji i ukazuje na značaj kontinuiranog praćenja AMR u različitim sistemima živinarske proizvodnje.