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INVESTIGATION ON ANTIMICROBIAL RESISTANCE RATES IN COMMENSAL *ESCHERICHIA COLI* ISOLATES FROM BROILERS ORIGINATING FROM BULGARIA (2020-2024)

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Abstract

The aim of the present study was to investigate the prevalence of resistance to several groups of antibiotics among commensal *Escherichia coli* bacteria isolated from broilers in Bulgaria. Between March 2020 and September 2024, a total of 510 cloacal swab samples were obtained from broilers on two poultry farms, one located in central Bulgaria and the other in the northern region of the country. Of the collected samples, 90 were taken from one-day-old broilers, 140 from 14-day-old broilers, and 280 from broilers aged 28 to 30 days. The total number of *Escherichia coli* isolates was 479, 89 of which from one-day-old broilers, 126 from 14-day-old and 264 from 28-30-day-old broilers. In addition, 12 samples were obtained from poultry litter, from which 10 *Escherichia coli* strains were isolated. The highest rates of resistance among commensal *Escherichia coli* from day-old birds were observed against ampicillin (49.4%), tetracycline (43.8%), followed by amoxicillin/clavulanic acid (34.8%). The strains resistant to the third-generation cephalosporins cefotaxime and ceftazidime were 7.9% and 3.4% respectively. The most prevalent resistance phenotype among the strains included resistance to aminopenicillins and tetracycline (28.1%), with the *tet*A gene being the most frequently detected (7.8%). The highest resistance rates were against ciprofloxacin (73.8%) in strains from 14-day-old broilers, while the highest resistance rate in isolates from 28-30-day-old birds was against ampicillin (65.6%). Tetracycline-resistant strains were 69.0% and 60.6%, respectively. The phenotypic profile including resistance to beta-

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lactams, tetracycline and ciprofloxacin predominated among 28.8% of the strains from both groups of broilers, with the highest prevalence for the *tet*A (26.4%) and *qnr*S (15.1%) genes. In the resistant strains isolated from poultry litter, the highest resistance rates were against ampicillin (100%), followed by tetracycline (80%), amoxicillin/clavulanic acid (70%) and ciprofloxacin (60%).

Key words: poultry, resistance, antimicrobial agents, commensal *Escherichia coli*

ISPITIVANJE STEPENA ANTIMIKROBNE REZISTENCIJE U KOMENSALNIM IZOLATIMA *ESCHERICHIA COLI* KOD PILIĆA BROJLERA IZ BUGARSKE (2020-2024)

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Kratak sadržaj

Cilj ovog rada je ispitivanje učestalosti rezistencije na nekoliko grupa antibiotika kod komensalinih bakterija *Escherichia coli* izolovanih kod brojlera u Bugarskoj. Između marta 2020. i septembra 2024., uzeto je 510 kloakalnih briseva od brojlera sa dve živinarske farme, jedne iz centralne Bugarske, a druge iz severnog dela zemlje. Od ukupnog broja uzoraka, 90 je od jednodnevnih pilića, 140 od brojlera uzrasta 14 dana, i 280 od pilića uzrasta 28 do 30 dana. *Escherichia coli* je izolovana iz 479 uzoraka: 89 od jednodnevnih brojlera, 126 od brojlera uzrasta 14 dana, 264 od brojlera uzrasta 28 do 30 dana. Dodatno, 10 *Escherichia coli* je izolovano iz 12 uzoraka prostirke. Najviše stope rezistencije kod komensalnih *Escherichia coli* kod jednodnvenih pilića zabeležene su na ampicilin (49,4%), tetraciklin (43,8%), a zatim amoksicilin/klavulansku kiselinu (34,8%). Sojevi rezistentni na cefotaksim i ceftazidim, cefalosporine treće generacije, činili su 7,9% odnosno 3,4% svih izolata. Najzastupljeniji fenotip rezistencije među sojevima obuhvatao je rezistenciju na aminopeniciline i tetraciklin (28,1%), pri čemu je gen *tetA* bio najčešće detektovan (7,8%). Najviša stopa otpornosti bila je na ciprofloksacin (73.8%) kod sojeva brojlera starih 14 dana, dok je najviša stopa kod izolata brojlera starih između 14 i 28 dana bila je na ampicillin

(65.6%). Stepen otpornosti na tetraciklin bila je 69.0% i 60.6%. Fenotipski profil koji uključuje rezistenciju na beta-laktame, tetraciklin i ciprofloksacin preovladavao je kod 28,8% sojeva iz obe grupe brojlera, pri čemu su najčešće bili detektovani geni *tet*A (26,4%) i *qnr*S. Kod rezistentnih sojeva izolovanih iz prosirke, najviše stope rezistencije zabeležene su prema ampicilinu (100%), zatim tetraciklinu (80%), amoksicilinu/klavulanskoj kiselini (70%) i ciprofloksacinu (60%).

Ključne reči: živina, otpornost, antimikrobni agensi, komensalna *Escherichia coli*

INTRODUCTION

The spread of antimicrobial resistance among bacteria is a significant contemporary public health issue, impacting both animal and human health, as well as the safety of foods of animal origin (EFSA, JIACRA IV, 2024). The selective pressure from the use of antibiotics in poultry farming is one of the essential factors affecting the emergence of resistance in bacteria (Agyare et al., 2018). This process poses risks to various ecological niches in nature beyond the animal population. Additionally, it facilitates the transfer of genetic determinants, contributing to the development of cross-resistance, the emergence of multidrug-resistant strains, and the presence of diverse genetic platforms enabling horizontal gene transfer of resistance traits to chemotherapeutics (Singer and Williams-Nguyen, 2014; Skarzyńska et al., 2020). Several studies advance the hypothesis about a link between the use of antibiotics in animal husbandry, including poultry farming, and the spread of resistance to chemotherapeutics among pathogenic bacterial strains isolated from humans (Silbergeld et al., 2008; Marshall and Levy, 2011; Aworth et al., 2020).

In line with Directive 2003/99/EC of the European Parliament and the Council, EU member states are required to submit annual data on the monitoring of resistance to chemotherapeutic agents in *Salmonella*, *Campylobacter*, methicillin-resistant staphylococci, and indicator *Escherichia coli* isolated from farm animals. From 2021, the requirements for testing resistance in indicator *E. coli* also apply to border control of imported fresh meat, with emphasis on the spread of *Salmonella* and *E. coli* strains producing extended-spectrum beta-lactamases (ESBL), AmpC beta-lactamases and carbapenemases, as some of key indicators of resistance rates in farm animals (ECDC, EFSA, 2020). The prevalence of co-resistance to the third-generation cephalosporins cefotaxime and ciprofloxacin among indicator animal *E. coli* and *Salmonella* spp. isolates is also considered a highly critical issue, given their importance in the therapy of severe bacterial infections in humans (EFSA, ECDC, 2024). The presumption about inclusion of indicator *E. coli* in monitoring systems and in various scientific studies largely relates to the fact that they are defined as reservoirs of genes in the intestinal microbiome, determining resistance to various chemotherapeutics, which may be transferred between different bacterial species causing infections in humans (EFSA, 2008, 2019).

Conversely, experts from the joint ECDC/EFSA/EMA report for the 2018–2021 period highlighted data showing a clear trend: the prevalence of cephalosporin-resistant indicator *E. coli* isolates producing extended-spectrum beta-lactamases in animals is directly linked to the use of cephalosporins in livestock farming (JIACRA IV, 2024). Their conclusion is similar regarding the use of aminopenicillins and the prevalence of resistant *E. coli* strains from animals. The report also indicates that penicillins (29%) and tetracyclines (23.6%) are the most widely used group of chemotherapeutic agents in animal.

MATERIAL AND METHODS

Materials and bacteriological examinations

Between March 2020 and September 2024, a total of 510 cloacal swab samples were collected from broilers at two poultry farms—one situated in central Bulgaria and the other in the northern region of the country. Twelve poultry litter samples were also collected. Of all samples, 90 were from one-day-old broilers, 140 from 14-day-old broilers and 280 from 28-30-day-old broilers.

For primary isolation of *E. coli* bacteria, swab samples were cultured in McConkey agar (Himedia, Biosciences, India) and incubated at 37 °C for 24 hours. The biochemical identification of *E. coli* was performed using Kligler iron agar (Himedia Biosciences, India), IMViC test (production of indole, methyl red test, Voges-Proskauer test, Simmons citrate agar) produced by Himedia, Biosciences, (India) and *Enterobacteriacae* identification kit (ENTERO test 24N, Erba Lachema, Czech Republic).

Methods for detection of *E. coli* strains' susceptibility to chemotherapeutics

The susceptibility of *E. coli* isolates to chemotherapeutic drugs was determined through the disk diffusion method and the following disks: ampicillin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cefotaxime (10 μg), ceftazidime

(5 μg), gentamicin (10 μg), tetracycline (30 μg) and ciprofloxacin (5 μg) (Himedia Biosciences, India). The chemotherapeutics' MICs were determined by the E-test (Hi Comb™, Himedia Biosciences, India). The MICs of gentamicin and tetracycline were determined using E-test (Liofilchem, MTS™, Italy). To identify the ESBL-producing *E. coli* strains, an E-test with a combination of ceftazidime/clavulanic acid (0.064-4 μg/mL) and cefotaxime/clavulanic acid (0.016-1 μg/mL) (Hi CombTM, Himedia Biosciences, India) was used. The interpretation of the results was based on the epidemiological cut-off (ECOFFs) values. The reference strain *E. coli* ATCC 25922 was used for control of antimicrobial susceptibility tests.

Genetic methods

The DNeasy Blood Tissue kit (Qiagen, Germany) was used for DNA extraction from pure cultures. The identification of genes conferring resistance to beta-lactams ($bla_{CTX\text{-}M\text{-}1}$, bla_{SHV}), tetracycline (*tet*A, *tet*B) and ciprofloxacin (*qnr*S) was performed with Microbial DNA qPCR assay kits (Qiagen, Germany). The thermal profile of the PCR reaction included denaturation at 95 °C for 10 min, 40 cycles with initial denaturation at 95 °C for 15 sec and amplification/elongation at 60 °C for 2 min. The amplification was performed in a Stratagene Mx3000PqPCR system (Agilent Technologies, USA).

RESULTS

A total of 479 *E. coli* isolates were obtained, including 89 from one-day-old broilers, 126 from 14-day-old broilers, and 264 from broilers aged 28–30-day-old. In addition, 12 samples were collected from poultry litter, from which 10 *E. coli* strains were isolated.

Table 1 presents the results on the sensitivity of the *E. coli* strains to chemotherapeutic agents tested by the disk diffusion method, while Tables 2 and 3 present the MIC values of the chemotherapeutics for isolates obtained from one-day-old broilers and from growing broilers, respectively. Most frequently, commensal *E. coli* bacteria from one-day-old broilers were resistant against ampicillin (49.4%), tetracycline (43.8%), and amoxicillin/clavulanic acid (34.8%). Among all isolates, 7.9% were resistant to the third-generation cephalosporin cefotaxime, and 3.4% showed resistance to ceftazidime. The highest resistance rates among strains from 14-day-old broilers were observed against ciprofloxacin (73.8%), while in isolates from 28–30-day-old broilers , the highest resistance was to ampicillin (65.6%). The prevalence rates of tetracycline-resistant strains were 69.0% and 60.6%, respectively. The resistance rates to cefotaxime and ceftazidime were 37.3%, 26.9% and 34.1%, 33.0%. The MIC_{90} of aminopenicillins, cefotaxime, gentamicin and ciprofloxacin in isolates from one-day-old broilers and from older broilers were similar – 16 μg/mL, 1 μg/mL, 2 μg/mL and 0.25 μg/mL, respectively. The respective MIC_{90} values of ceftazidime were 1 μg/mL and 2 μg/mL, and of tetracycline – 16 μg/mL and 32 μg/mL (Tables 2 and 3).

Table 1. Resistance among commensal *E. coli* strains isolated from broilers (n = 479)

	Isolates from broilers (one-day-old) n=89		Isolates from broilers (14-day-old) n= 126		Isolates from broilers (28-30-day-old) n=264		Total n=479	
Antimicrobial agents	**Number (%)**	**Confidence limits (CL)**	**Number (%)**	**Confidence limits (CL)**	**Number (%)**	**Confidence limits (CL)**	**Number (%)**	**Confidence limits (CL)**
Ampicillin	44 (49.4%)	39.1÷60.2	77 (61.1%)	52.5÷69.4	173 (65.5%)	59.8÷71.1	294 (61.4%)	57.0÷65.7
Amoxicillin/clavulanic acid	31 (34.8%)	25.3÷44.9	64 (50.8%)	42.1÷59.4	154 (58.3%)	52.3÷64.1	249 (52.0%)	47.5÷56.4
Cefotaxime	3 (3.4%)	0.6÷8.1	47 (37.3%)	29.1÷45.8	71 (26.9%)	21.7÷32.4	121 (25.3%)	21.5÷29.2
Ceftazidime	7 (7.9%)	3.2÷14.3	43 (34.1%)	26.2÷43.9	87 (33.0%)	27.5÷38.7	137 (28.6%)	24.7÷32.7
Gentamicin	17 (19.1%)	11.7÷27.8	36 (28.6%)	21.1÷36.7	63 (23.9%)	19.0÷29.2	116 (24.2%)	20.5÷28.1
Tetracycline	39 (43.8%)	33.7÷54.1	87 (69.0%)	60.7÷76.7	160 (60.6%)	54.6÷66.3	286 (59.7%)	55.3÷64.0
Ciprofloxacin	16 (18.0%)	10.7÷26.5	93 (73.8%)	65.8÷81.0	134 (50.5%)	44.5÷56.5	243 (50.7%)	46.2÷55.1

Table 2. MIC (μg/mL) of tested antimicrobial drugs in commensal *E. coli* isolates from one-day-old broiler (n = 89)

Antimicrobial agents	MIC_{90}	0.06	0.125	0.250	0.5	1	2	4	8	16	32	64	128
Ampicillin	16						2	10	33*	35	7	2	
Amoxicillin/ clavulanic acid	16					1	5	24	28*	21	4	5	1
Cefotaxime	1				4	82	3*						
Ceftazidime	1					82	5	2*					
Gentamicin	2				19	43	10*	17					
Tetracycline	16							47	3*	16	21	1	1
Ciprofloxacin	0.25	7	17	49	16*								

Legend: MIC thresholds are marked with asterisks

Table 3. MIC (μg/mL) of tested antimicrobial drugs in commensal *E. coli* isolates from 14-30-day-old broilers (n = 390)

Antimicrobial agents	MIC_{90}	0.06	0.125	0.250	0.5	1	2	4	8	16	32	64	128	256
Ampicillin	16						4	25	111*	189	37	19	5	
Amoxicillin/ clavulanic acid	16						7	11	154*	41	154	14	7	2
Cefotaxime	1				15	257	114*	4						
Ceftazidime	2				19	241	18	112*						
Gentamicin	2				19	93	179*	99						
Tetracycline	32							23	120*	157	37	50	1	2
Ciprofloxacin	0.25	3	21	139	215*	12								

Legend: MIC thresholds are marked with asterisks

Table 4 presents the phenotypic resistance profiles of *E. coli* strains isolated from both one-day-old and growing broilers, along with selected genetic determinants associated with resistance to beta-lactams, tetracycline, and ciprofloxacin. The predominant phenotype profile among resistant isolates from one-day-old broilers included aminopenicillins and tetracycline (28.1%), with the *tet*A gene (7.8%) being predominant. The phenotype profile including resistance to beta-lactams, tetracycline and ciprofloxacin was dominant among of strains isolated from growing broilers (28.8%), with the highest prevalence of the *tet*A (26.4%) and *qnr*S (15.1%) genes. The prevalence rates of the $bla_{CTX\text{-}M\text{-}1}$ and the *tet*B genes were 1.8% and 1.3%, respectively. The bla_{SHV} gene was not detected in any of the isolates resistant to beta-lactams from broilers of different age categories and from poultry litter.

Table 4. Phenotype resistance profiles and genes of antimicrobial resistance in commensal *E. coli* isolates from broiler chickens

Phenotype profiles	**Genes of antimicrobial resistance**				
	$\boldsymbol{bla}_{CTX\text{-}M\text{-}1}$	$\boldsymbol{bla}_{SHV}$	***tet*A**	***tet*B**	***qnr*S**
One-day-old broilers (n = 89)					
CIP (n = 3)	-	-	-	-	-
G (n = 6)	-	-	-	-	-
Amp, G CIP (n = 5)	-	-	-	-	-
Amp, T, CIP (n = 5)	-	-	2 (2.2%)	-	1 (1.1%)
Amp, AMC, T (n = 25)	-	-	5 (5.6%)	-	-
Amp, AMC,CAZ, T (n = 3)	-	-	-	-	-
Amp, AMC, CTX, G, T (n = 3)	-	-	-	-	-

Phenotype profiles	**Genes of antimicrobial resistance**				
	bla$_{CTX-M-1}$	***bla***$_{SHV}$	***tet*A**	***tet*B**	***qnr*S**
Amp, G, T, CIP (n=3)	-	-	-	-	2 (2.2%)
14-30-day- old broilers (n = 390)					
T (n = 24)	-	-	6 (1.5%)	-	-
CIP (n = 6)	-	-	-	-	-
T, G (n = 67)	-	-	49 (12.6%)	-	-
AMP, G, T, CIP (n = 3)	-	-			
AMP, CAZ, G,T (n = 19)	-	-	3 (0.8%)	-	-
AMP, CTX, CAZ, G, T (n = 10)	2 (0.5%)	-	4 (1.0%)	3 (0.8%)	-
AMP, AMC, CTX,CIP (n = 105)	15 (3.8%)	-	-	-	73 (18.7%)
AMP, AMC, CTX, CAZ, T, CIP (n = 113)	7 (1.8%)	-	103 (26.4%)	5 (1.3%)	59 (15.1%)
Total (n = 479)	**24 (5.0%)**	-	**172 (36.0%)**	**8 (1.7%)**	**135 (28.2%)**

Legend: AMP-ampicillin, AMC-amoxicillin/clavulanic acid, CTX- cefotaxime, CAZ-ceftazidime, G- gentamicin, T- tetracycline, CIP- ciprofloxacin

The results about the susceptibility of the poultry litter isolates to the studied antibiotics and their phenotypic profiles are presented in Tables 5 and 6. In the resistant poultry litter strains, the highest rates were against ampicillin (100%), tetracycline (80%), amoxicillin/clavulanic acid (70%) and ciprofloxacin (60%). The most common phenotype profile of resistance included beta-lactams, tetracycline and ciprofloxacin (40%), with the *qnr*S gene being observed in 40% of the strains and the *tet*A gene in 50% of the resistant *E. coli* bacteria. The MIC_{90} values for *E. coli* isolates from poultry litter were 16 µg/

mL for aminopenicillins and tetracycline, 2 µg/mL for gentamicin and third-generation cephalosporins, and 0.25 µg/mL for ciprofloxacin.

Table 5. Resistance among commensal *Escherichia coli* strains isolated from poultry litter (n = 10)

Antimicrobial agents	**Isolates from poultry litter (n = 10) Number (%)**	**Confidence limits (CL)**	**MIC_{90}**
Ampicillin	10 (100%)	90.7÷100	16
Amoxicillin/clavulanic acid	7 (70.0%)	40.7÷92.8	16
Cefotaxime	2 (20.0%)	2.3÷48.7	1
Ceftazidime	4 (40.0%)	13.4÷70.2	2
Gentamicin	6 (60.0%)	44.1÷86.5	2
Tetracycline	8 (80.0%)	51.2÷97.6	16
Ciprofloxacin	6 (60.0%)	44.1÷86.5	0.25

Table 6. Phenotype resistance profiles and genes of antimicrobial resistance in commensal *E. coli* isolates from poultry litter

Phenotype profiles	**Genes of antimicrobial resistance**				
	***bla*$_{CTX-M-1}$**	***bla*$_{SHV}$**	***tet*A**	***tet*B**	***qnr*S**
	Poultry litter (n=10)				
AMP, G, T (n=2)		-	2 (20%)	-	-
AMP, AMC, T, CIP (n=4)		-	2 (20%)	-	3 (30%)
AMP, AMC, CAZ, G, T (n=1)	1 (10%)	-	1 (10%)	-	-
AMP, CAZ, G, T (n=1)	1 (10%)	-	-	1 (10%)	-
AMP, AMC, CTX, CAZ, G CIP (n=2)	2 (20%)	-	-	-	1 (10%)
Total (n=10)	**4 (40.0%)**	**-**	**5 (50.0%)**	**1 (10%)**	**4 (40%)**

Legend: AMP-ampicillin, AMC-amoxicillin/clavulanic acid, CTX-cefotaxime, CAZ- ceftazidime, G- gentamicin, T- tetracycline, CIP- ciprofloxacin

DISCUSSION

In the present study, 28.1% of the resistant *E. coli* strains from one-day-old chickens exhibited a phenotypic resistance profile that included both ampicillin and tetracycline. Overall, resistance to ampicillin was observed in 49.4% of the isolates, while 43.8% were resistant to tetracycline. According to Nilsson et al. (2014) and Projahn et al. (2018), the emergence of resistance in *E. coli* bacteria isolated from day-old broilers may be a consequence of vertical transfer of genetic factors during incubation and transport. In 28.8% of the resistant *E. coli* isolates from 14-30-day-old broilers, the phenotype profile included ampicillin, tetracycline and ciprofloxacin, with the highest resistance rate observed against ciprofloxacin (73.8%) in strains from 14-day-old broilers, and against ampicillin (65.6%) in those isolated from 28-30-day-old broilers. Chuppava et al. (2019) and Luiken et al. (2020), hypothesized that the environment in poultry farms, e.g. poultry litter and dust, played a major role in the spread of resistant *E. coli* in growing broilers. In their experimental study Montoro-Dasi et al. (2021) also reported a wider spread of penicillin- and ciprofloxacin-resistant *E. coli* isolates from growing broilers. The authors affirmed that the presence of strains resistant against third-generation cephalosporins was mainly based on environmental horizontal gene transfer and defined this circumstance as critical for the breeding of broiler birds. On the other hand, WHO experts (2017) placed an emphasis on the speculative opinion about a possible risk associated with the transmission of environmental genetic factors determining resistance to fluorinated quinolones in commensal intestinal bacteria, and the thesis that the risk was mainly related to the selective pressure from their use in poultry farming. In the Netherlands, Hesp et al. (2019) conducted a comparative analysis of resistance profiles in commensal *E. coli* strains isolated from broilers before and after 2009. They noted that despite the implementation of restrictive policies on the use of fluoroquinolones in poultry farming after 2009, resistance rates to ciprofloxacin in *E. coli* remained high. The authors attributed this finding on the fact that the observed resistance against ciprofloxacin was not plasmid-determined, unlike the genetic background of resistance against third-generation cephalosporins. In our study, alongside the observed high resistance rate to ciprofloxacin (73.8%) in *E. coli* strains from growing broilers, the plasmid-mediated *qnr*S gene was detected in 34% of the resistant strains and in 40% of the isolates from poultry litter.

In comparison to our findings, Racewicz et al. (2022) reported higher resistance rates in *E. coli* strains from broilers in Poland, with 100% resistance to ampicillin and 92% to ciprofloxacin. The authors underlined the high resistance against ampicillin (100%) and doxycycline (100%) in strains isolated from poultry litter. However, the genetic profile of the beta-lactam-resistant strains showed a low prevalence of ESBL genes, with the bla_{CTX-M} gene detected in only one *E. coli* strain (4%). Their results were comparable regarding the prevalence of the *qnr*S gene in ciprofloxacin-resistant *E. coli* bacteria. Regarding doxycycline-resistant *E. coli*, the *tet*A and *tet*B genes were detected in 36% of strains from broilers and in 37.5% of strains isolated from poultry litter. The presence of the *tet*A gene predominated in 75% of the strains from poultry litter and in 52% of isolates from broilers. In this regard, our results also showed a wider spread of the *tet*A gene compared to that of the *tet*B gene in *E. coli* isolates from both broilers (36%; 1.7%) and poultry litter (50%; 10%).

Over the past years, multidrug-resistant commensal *E. coli* producers of ESBLs have been recognized as a specific and important indicator of the spread of resistance to chemotherapeutics in various public health sectors. The plasmid-mediated genes conferring resistance to extended-spectrum beta-lactams are often linked to co-selection mechanisms that facilitate the transfer of resistance to other classes of chemotherapeutic agents. This, combined with selective pressure from drug use, poses a significant risk to various public health sectors (Schink et al., 2013; Poirel et al., 2018). In this context, a collective mechanism involving plasmid-encoded efflux pump genes—contributing to resistance against fluoroquinolones and other antibiotic classes—may also be involved. Efflux pump mechanisms also play a key role in the development of resistance to tetracyclines. Since tetracyclines are among the most commonly used antimicrobials in livestock and poultry farming, the associated risks are amplified by the strong selective pressure resulting from their widespread use.

CONCLUSION

As previously mentioned, this study demonstrated the prevalence of multidrug-resistant phenotypic profiles including resistance to beta-lactams, tetracycline, and ciprofloxacin in *E. coli* isolates from both growing broilers and poultry litter. Strains isolated from one-day-old broilers predominantly showed resistance to aminopenicillins and tetracycline. Concerning the diversity of genetic mechanisms and factors influencing resistant *E. coli* as a specific reservoir within the intestinal microbiome of broiler chickens, the major trends observed in this four-year study align with predicted patterns of resistance spread in poultry reported in other European countries. On the other hand, despite the restrictive antibiotic policies implemented at the two studied farms, the possibility remains that factors beyond selective pressure in poultry farming contribute to the spread of such resistant strains across different public health sectors.

Author's Contribution:

VU contributed to the conceptualization and design of the study, participated in the investigation, methodology, supervision, and was involved in writing, reviewing, and editing the manuscript. RS participated in the investigation, contributed to methodology and resources, and was involved in drafting the manuscript. DD contributed to the investigation, methodology, and provision of resources. All authors read and approved the final manuscript.

Competing interest

The authors declare no conflict of interest related to the present study.

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