Phylogenetic Characterization of *Escherichia coli* Isolates Obtained Before and After Vaccination from Broilers with Colibacillosis

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ABSTRACT

Application of commercial attenuated live *Escherichia coli* vaccine to broiler flocks may be associated with changes in the phylogenetic distribution of avian pathogenic E. coli (APEC) isolates, potentially decreasing the prevalence of highly pathogenic phylogroups while increasing the proportion of low-pathogenic groups. The aim of this study was to examine the changes in phylogenetic groups of APEC strains isolated from broiler flocks vaccinated with commercial attenuated live *E. coli* vaccine, to evaluate the potential effects of the vaccine on phylogenetic diversity and to compare the antibiotic resistance profiles of APEC isolates obtained at early and late stages of broiler life. The study material consisted of 360 *E. coli* isolates, 180 before vaccination and 180 after vaccination, from 15 farms throughout 2022. Bacterial identification and antibiotic susceptibility tests were performed using the Gram-negative identification card (NMIC/ID-433) in the automated microbiology system (BD Phoenix 100™). Phylogenetic grouping of the isolates was performed using the Clermont multiplex PCR method targeting the genes. The distribution of phylogenetic groups before and after vaccination was compared statistically using the chi-square ($\chi 2$) test. In the pre-vaccination period, it was observed that the majority of the isolates were concentrated in groups B1 (22.8%), F (21.7%) and E (21.1%). In the post-vaccination period, it was determined that groups B2, C and D had completely disappeared and there was a significant increase in the rate of group A (56.7%). The isolates exhibited high resistance rates to penicillin (70%) and tetracycline group antibiotics (78%), and the multiple antibiotic resistance rate reached 80%. This study showed that the isolates had high resistance rates to commonly used antibiotics, but low resistance levels were observed to clinically critical antibiotics such as colistin. In addition, it was observed that commercial live attenuated *E. coli* vaccine was associated with notable changes in the distribution of phylogenetic groups, particularly reducing the prevalence of high-virulence groups (B2, C, D), while increasing group A.

Keywords: Antibiotic resistance; broiler; *Escherichia coli*; phylotyping; live vaccine.

INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC), an extraintestinal pathogenic *E. coli* (ExPEC), causes various local and systemic infections (perihepatitis, air sacculitis, pericarditis, egg peritonitis, salpingitis, coligranuloma, omphalitis, cellulitis, osteomyelitis, arthritis) in chickens, turkeys, ducks and many other poultry species, and these infections are generally called poultry colibacillosis (1,2). Avian colibacillosis is one of the leading causes of death and morbidity in poultry, with mortality rates reaching up to 20% and significant morbidity (3).

APEC is genetically similar to ExPEC strains that cause serious diseases in humans and can transfer resistance genes to human pathogens through horizontal gene transfer (4), leading to an increase in foodborne zoonotic infections and the spread of antibiotic resistance that threatens public health (5).

Phylogenetic grouping is an important tool in the prevention of infections and the development of treatment methods by determining the genetic characteristics of APEC strains (6,7). *E. coli* was first classified into four main phylogroups (A, B1, B2 and D) by the triplex PCR method developed by Clermont *et al.* (2000) (6). Subsequent studies expanded this classification and defined a total of eight phylogroups (A, B1, B2, C, D, E, F, *Escherichia* cryptic group I) (7). Strains of phylogroups B2 and D are generally known as extraintestinal pathogenic *E. coli* (ExPEC), while isolates A and B1 are considered commensal. There is limited information on the pathogenicity of strains of phylogroups C, E and F (6,7).

Although the use of antimicrobial drugs is a common practice in the treatment of ExPEC infections, this situation leads to the emergence of multidrug-resistant strains. Cephalosporins, quinolones, tetracyclines and trimethoprim-sulfamethoxazole are among the antibiotics frequently used in poultry farms and an increase the incidence of strains resistant to these antibiotics (8).

The diversity of APEC serotypes in colibacillosis cases limits the effectiveness of current vaccines, and the lack of an effective vaccine against APEC infections makes control of this pathogen difficult (9). As of today, two commercial vaccines are available: live attenuated Poulvac® *E. coli* and inactivated Nobilis® *E. coli* vaccines. However, there is no definitive information on the effectiveness of these limited options in preventing infections in broilers (1).

While vaccines are used to reduce colibacillosis, little is known about their effects on the genetic diversity and phylogenetic distribution of APEC, which are critical to understanding vaccine efficacy. Application of live vaccines may affect the phylogenetic distribution of APEC isolates and the severity of infections depending on this distribution. The aim of this study was to examine the changes in phylogenetic groups of APEC strains isolated from broiler flocks vaccinated with a commercial attenuated live *E. coli* vaccine, to evaluate the potential effects of the vaccine on phylogenetic diversity and to compare the antibiotic resistance profiles of APEC isolates obtained at early and late stages of broiler life.

MATERIAL AND METHODS

Ethical Statement

This study was conducted with the approval of the Local Ethics Committee for Animal Experiments of Aydın Adnan Menderes University (ADU-HADYEK), dated, 29.09.2021, numbered 64583101/2021/141.

Material

The material of this study consisted of a total of 360 *E. coli* isolates, 180 before vaccination and 180 after vaccination, from 15 different farms of a broiler farm during 2022. *E. coli* isolates were obtained from morphologically altered livers after necropsies performed in farm laboratories. A total of 12 isolates obtained from broilers on each farm were delivered to Aydın Adnan Menderes University University Faculty of Veterinary Medicine Department of Microbiology Laboratories under cold chain conditions. The flocks on the farms were vaccinated with an attenuated live commercial vaccine (Poulvac® *E. coli* vaccine) using the spray method on the 5th day of their lives.

Bacterial Isolation

E. coli isolates were inoculated on MacConkey (Merc, Germany) agar and left for incubation. After inoculation, the petri dishes were incubated for 18-24 hours at 37˚C under aerobic conditions. One of the pink colonies fermenting lactose on MacConkey agar was selected and passaged onto Eosin Methylene Blue agar (Merc, Germany). It was kept on EMB agar under the same incubation conditions at 37˚C for 18-24 hours. Colonies showing metallic green color on EMB agar were subjected to Gram staining and biochemical tests (oxidase, catalase, indole). Isolates that were Gram negative rod-shaped, oxidase negative, and catalase and indole tests positive were considered suspicious for *E. coli* (10). These isolates were stored in Brain Heart Infusion Broth (Merc, Germany) with 20% glycerol at -20˚C.

Identification and Antibiotic Susceptibility Tests

With the BD Phoenix 100™ NMIC/ID-433 kit, isolates were tested against 20 antibiotics belonging to nine antimicrobial families (aminoglycoside (AG): amikacin (AN), gentamicin (GM); carbapenem: ertapenem (ETP), imipenem (IMP), meropenem (MEM).; cephalosporin (CP): cefazolin (CFZ), cefuroxime (CXM), ceftazidime (CAZ), ceftriaxone

Table 1. Primers used in the study.

Primer	Target gene	Sequence (5'-3')	Amplicon size (bp)	T_m
*chu*A.1b	*chu*A	ATGGTACCGGACGAACCAAC	288	60.5
*chu*A.2		TGCCGCCAGTACCAAAGACA		60.5
*yja*A.1b	*yja*A	CAAACGTGAAGTGTCAGGAG	211	58.4
*yja*A.2b		AATGCGTTCCTCAACCTGTG		58.4
*TspE*4C2.1b	*TspE*4.C2	CACTATTCGTAAGGTCATCC	152	56.4
*TspE*4C2.2b		AGTTTATCGCTGCGGGTCGC		62.5
*Ace*K F	*arp*A	AACGCTATTCGCCAGCTTGC	400	60.5
*Arp*A1 R		TCTCCCCATACCGTACGCTA		60.5
*Arp*AgpE F	*arp*A	GATTCCATCTTGTCAAAATATGCC	301	60.1
*Arp*AgpE R		GAAAAGAAAAAGAATTCCCAAGAG		58.4
*trp*AgpC.1	*trp*A	AGTTTTATGCCCAGTGCGAG	219	58.4
*trp*AgpC.2		TCTGCGCCGGTCACGCCCC		68.1
*trp*BA.F	*trp*A	CGGCGATAAAGACATCTTCAC	489	59.4
*trp*BA.R		GCAACGCGGCCTGGCGGAAG		68.7

(CRO), cefepime (FEP); penicillin (P): ampicillin (AMP); β Lactam (BL): ceftolozane tazobactam (CT), amoxicillin clavulanate (AXC), ampicillin sulbactam (AS), piperacillin tazobactam (TZP); lipopeptide (LP): colistin (COL); folate (F): trimethoprim sulfamethoxazole (SXT); quinolone (K): ciprofloxacin (CIP), levofloxacin (LF); tetracycline (T): tigecycline (TGC)) resistance status is being evaluated. In the test, *E. coli* ATCC 25922 strain was used as quality the control strain. EUCAST (2022) human clinical breakpoints were used for interpretation, as specific veterinary breakpoints were not available for all tested antibiotics (11). Isolates resistant to three or more antibiotic classes were classified as multiple drug resistant (MDR) (12).

DNA Extraction

Sonication method was used for DNA extraction (13). Purity and quantity were assessed via a nanodrop (Maestrogen, Taiwan), with an OD260/OD280 ratio of 1.6-2.0 (14).

Primers

Phylogroup distribution of *E. coli* isolates were determined by multiplex polymerase chain reaction method by targeting *chu*A, *yja*A, *TspE*4.*C*2, *arp*A genes (7). Phylogroup classification was performed by targeting *trp*A (15). and *arp*A genes in the definition of group C and group E (16). *E. coli* was classified into eight different phylogroups (A, B1, B2, C, D, E, F, clade I) using target genes (7). Phylogenetic distribution was determined using the quadruplex PCR method (7,15,16) (Table 1).

Polymerase Chain Reaction

PCRs were performed in 25 µl volumes with final concentrations: 1x Taq enzyme buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.4 ρmol primers, and 1.5 U Taq DNA polymerase (Fermentas, USA). PCR tubes were prepared with 22 µl of master mix and 3 µl of DNA for each sample. Amplification involved initial denaturation at 95°C for 5 min, 30 cycles of 95°C for 30 sec, annealing at 56°C (*chu*A, *yja*A, *tsp*E4.*C*2, *arp*A, *trp*A) for 30 sec, 72°C for 60 sec, and a final extension at 72°C for 10 min. *E. coli* ATCC 25922 served as the positive (internal) control, and *S*. Enteritidis ATCC 13076 as the negative control. Target genes producing a single band of the expected size upon amplification were considered positive.

Statistical Analysis

For statistical analysis, Statistical Package for Social Sciences (SPSS) version 23.0 (SPSS Inc., USA) was used. Pearson's chi-square ($\chi 2$) test (Fisher's Exact $\chi 2$ Test) compared frequency data. These analyses were performed to evaluate the effects of commercial *E. coli* vaccine applied to broiler flocks on phylogenetic diversity. The changes in phylogenetic groups of isolates obtained before and after vaccination were compared to examine the effect of the vaccine on phylogenetic diversity and whether there was a significant change in the

Table 2. Antibiotic resistance status of isolates.

Antimicrobial family	Antibiotic	Days		All isolates (N=360) N (%)
		0-5.	35-42.	
		(N=180) N (%)	(N=180) N (%)	
Aminoglycoside	Amikacin	0 (0)	0 (0)	0 (0)
	Gentamicin	22 (12)	81 (45)	103 (29)
Carbapenem	Ertapenem	9 (5)	8 (4)	17 (5)
	Imipenem	0 (0)	0 (0)	0 (0)
	Meropenem	0 (0)	0 (0)	0 (0)
Cephalosporin	Cefazolin	23 (13)	38 (21)	61 (17)
	Cefuroxime	26 (14)	38 (21)	64 (18)
	Cefotazidime	16 (9)	29 (16)	45 (13)
	Ceftriaxone	15 (8)	29 (16)	44 (12)
	Cefepim	17 (9)	28 (16)	45 (13)
Penicillin	Ampicillin	114 (63)	137 (76)	251 (70)
Beta lactam	Ceftolozane tazobactam	2 (1)	9 (5)	11 (3)
	Amoxicillin clavulanate	58 (32)	83 (46)	141 (39)
	Ampicillin sulbactam	46 (26)	77 (43)	123 (34)
	Piperacillin tazobactam	24 (13)	34 (19)	58 (16)
Polymyxin	Colistin	2 (1)	7 (4)	9 (3)
Folate	Trimethoprim sulfamethoxazole	55 (31)	96 (53)	151 (42)
Quinolone	Ciprofloxacin	75 (42)	112 (62)	187 (52)
	Levofloxacin	46 (26)	107 (59)	153 (43)
Tetracycline	Tigecycline	116 (64)	164 (91)	280 (78)

prevalence of phylogenetic groups. Results were evaluated at a 95% confidence interval and with P<0.05 were considered statistically significant.

RESULTS

Isolation and Identification

In this study, a total of 360 *E. coli* isolates were obtained from 15 different broiler farms, 12 isolates each before and after vaccination. During the identification process, Gram negative rod-shaped bacteria with metallic green sheen on Eosin Methylene Blue agar, pink colonies on MacConkey agar, oxidase negative, catalase positive and indole positive properties were evaluated as suspicious isolates for *E. coli*. Then, a total of 360 *E. coli* suspected isolates were analysed with NMIC/ID 433 panels using the BD Phoenix 100™ automated microbiology system and identified as *E. coli*.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of a total of 360 isolates obtained within the scope of the study was performed with NMIC/ID 433 panels using the BD Phoenix 100™ automated microbiology system. According to the antibiogram analyses performed on the isolates before (0-5. days) and after (35-42. days) vaccination, the resistance rates were determined as follows (Table 2, Fig. 1). The results show that resistance rates are moderately high, especially against penicillin and tetracycline group antibiotics, while it is noteworthy that resistance rates against critical antibiotics such as colistin remained at low levels.

MDR:

The findings show that 53.8% (97/180) of the isolates obtained up to the fifth day of broiler life and 81.1% (146/180) of the isolates obtained when they were 35-42 days old

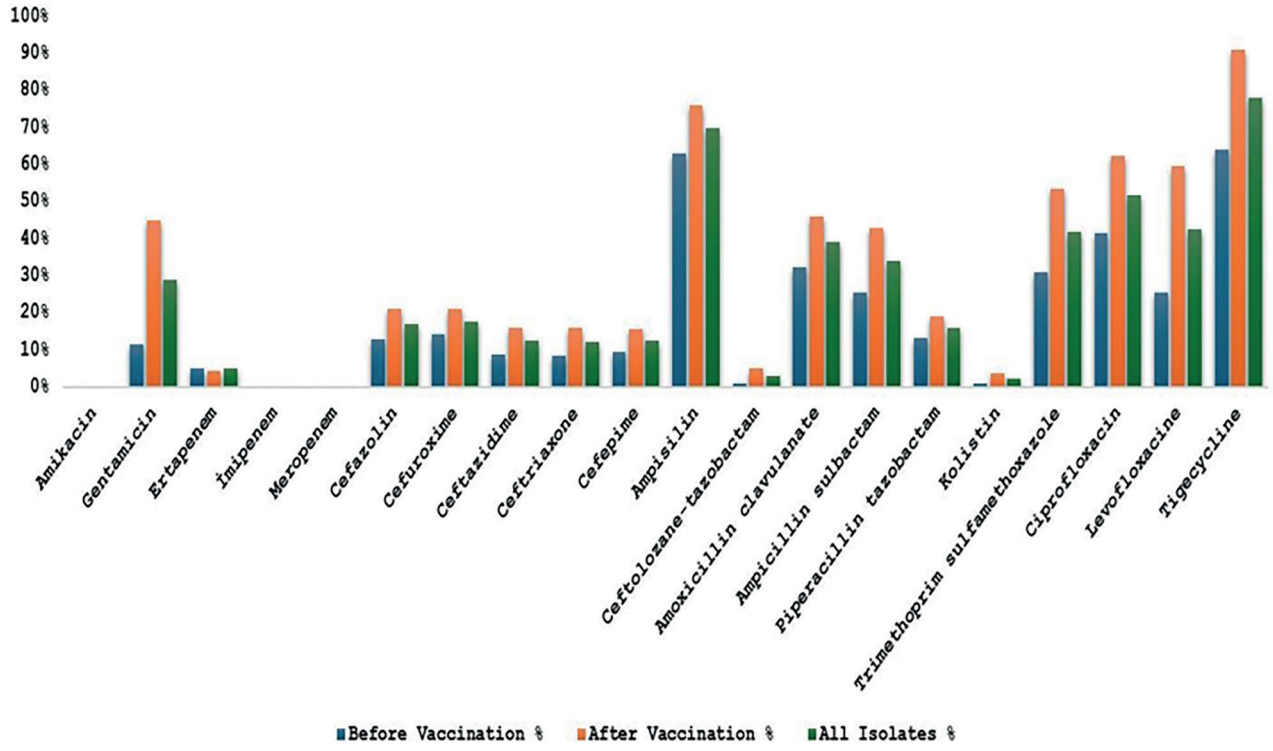

Figure 1. Resistance status of *E. coli* isolates to antibiotics

were resistant to multiple antibiotics. All of isolates 67.5% (243/360) were resistant to multiple antibiotics (Table 3, Figure 2).

Phylotyping

In the pre-vaccination period, the majority of isolates were concentrated in groups B1 (22.8%), F (21.7%) and E (21.1%). Vaccination had particularly reduced the prevalence of high (B2) or moderate (C, D) virulence phylogenetic groups, causing the low-virulence group A to become dominant. This change suggests a shift in phylogenetic group distribution following vaccination, but further studies are needed to assess its impact on herd health (Table 4, Figure 3, Figure 4).

Statistical Analysis

The findings revealed that commercial live attenuated *E. coli* vaccine caused significant changes in the prevalence of phylogenetic groups and had a significant effect on all phylogroups (A, B2, C, D, E, F) except phylogroup B1 *(*Table 5*)*.

DISCUSSION

Although poultry production is a rapidly growing sector worldwide, viral and bacterial infections significantly negatively affect this sector (18). APEC is seen as a widespread infectious agent in different continents such as Europe, Asia, America, Africa and Australia (1,2), as well as in our country (19), and causes serious economic losses. Although APEC is generally considered a secondary pathogen, it can lead to high mortality rates due to its virulence factors (2). At the same time, the fact that it acts as a reservoir of resistance genes makes this bacterium important not only in terms of animal health but also in terms of public health as a zoonotic threat (1,2). The aim of this study was to examine the changes in phylogenetic groups of APEC strains isolated from broiler flocks vaccinated with commercial attenuated live *E. coli* vaccine, to evaluate the potential effects of the vaccine on phylogenetic diversity and to compare the antibiotic resistance profiles of APEC isolates obtained at early and late stages of broiler life. Such studies contribute to the development of infection control strategies; provide important information in terms of reducing economic losses and protecting public health.

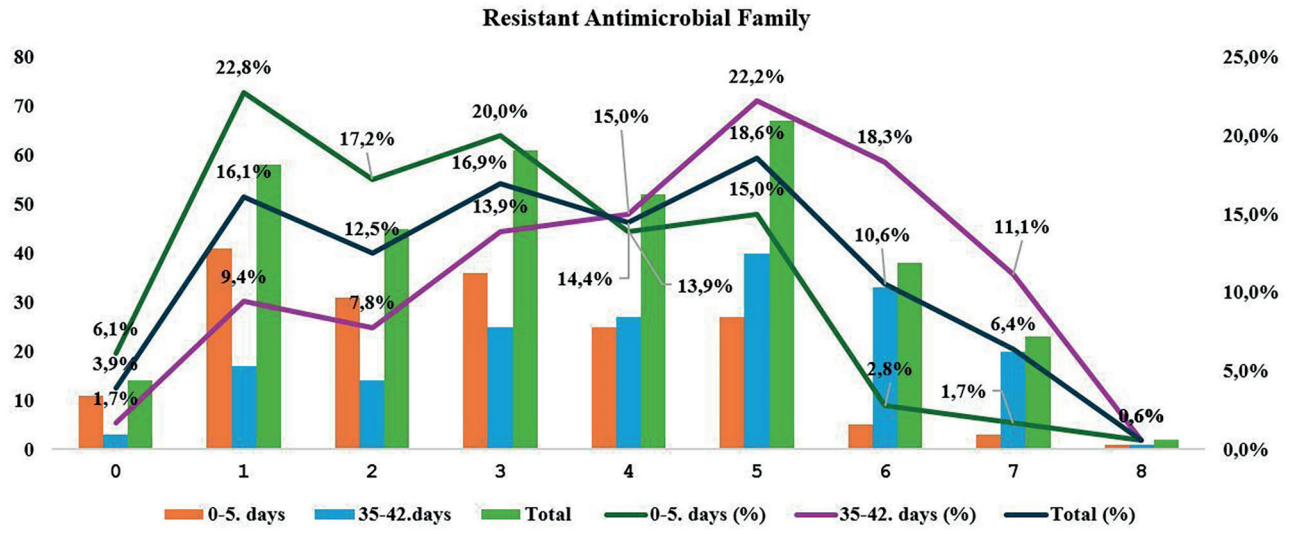

Figure 2. Multiple antibiotic resistance status before and after vaccination.

Table 3. Comparison of multiple antibiotic resistance status before and after vaccination.

Number of resistant antimicrobial families	0-5. days (N=180) N (%)	35-42. days (N=180) N (%)	Total (N=360) N (%)
0	11 (6.1)	3 (1.7)	14 (3.9)
1	41 (22.8)	17 (9.4)	58 (16.1)
2	31 (17.2)	14 (7.8)	45 (12.5)
3	36 (20.0)	25 (13.9)	61 (16.9)
4	25 (13.9)	27 (15.0)	52 (14.4)
5	27 (15.0)	40 (22.2)	67 (18.6)
6	5 (2.8)	33 (18.4)	38 (10.6)
7	3 (1.7)	20 (11.1)	23 (6.5)
8	1 (0.5)	1 (0.5)	2 (0.5)

In this study, antibiotic resistance profiles of *E. coli* isolates from broilers were evaluated. In particular, isolates obtained from early life stages (first 5 days) and near-slaughter periods (35-42 days) of broilers were compared, and the trend in resistance profiles over time was analysed. The resistance rate detected for gentamicin in our study (29%) was higher than the resistance rate reported in a study conducted in Ukraine (14%) (20). In contrast, lower resistance rates for beta-lactam/beta-lactamase inhibitor combinations (e.g. amoxicillin-clavulanate) (39%) were significantly different from the higher rates in the Ukrainian study (69%). Ampicillin resistance from the penicillin group was detected as 70% in our study, which is similar to amoxicillin resistance reported in Ukraine (78%) (20). The resistance rate to tigecycline, a tetracycline antibiotic, was found to be the highest in our study at 78%. There is no licensed veterinary drug formulation for the use of tigecycline in poultry. The high resistance rate to tigecycline observed in this study is not attributable to direct use of this antibiotic in poultry, as it is not licensed for such use. Instead, it is likely related to co-selection mechanisms linked to the widespread use of other tetracycline-class antibiotics, such as oxytetracycline or doxycycline, which can promote the persistence of tetracycline resistance genes (e.g., tetA, tetB). The long-term and widespread use of tetracycline antibiotics in poultry in Türkiye world may contribute to the development of these resistance. In the study conducted in Ukraine (20), high levels of resistance rates were reported for oxytetracycline (75%) and doxycycline (58%). Previous studies have shown that tetracycline resistance is associated with the presence of *tet*A and *tet*B genes and that these genes

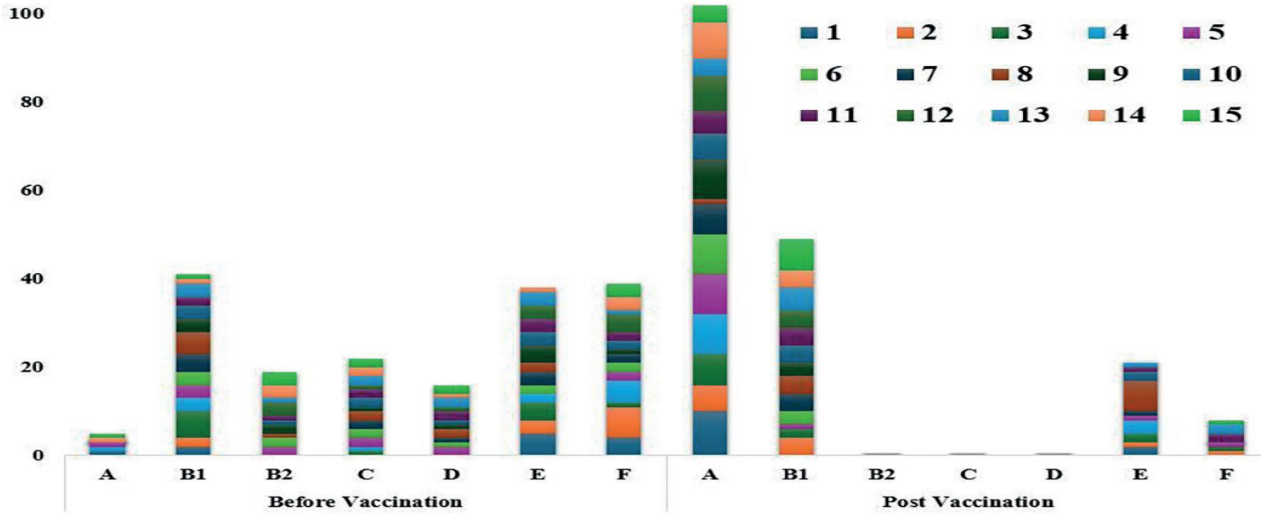

Figure 3. Phylotype numbers on farms before and after vaccination

Table 4. Phylogenetic distribution before and after vaccination.

Phylogenetic group	Before vaccination (N=180) N (%)	After vaccination N=180, (%)	Total N=360, (%)
A	5 (2.8)	102 (56.7)	107 (29.7)
B1	41 (22.8)	49 (27.2)	90 (25.0)
B2	19 (10.6)	0 (0)	19 (5.3)
C	22 (12.2)	0 (0)	22 (6.1)
D	16 (8.9)	0 (0)	16 (4.4)
E	38 (21.1)	21 (11.7)	59 (16.4)
F	39 (21.7)	8 (4.4)	47 (13.1)

are widely detected worldwide (21). This situation can be attributed to the uncontrolled use of tetracyclines. In our study, the resistance rates of ciprofloxacin (52%) and levofloxacin (43%) from the quinolone group antibiotics are remarkable. In the study conducted in Ukraine (20), resistance rates for enrofloxacin and flumequine were reported as 56% and 78%, respectively. It is reported that ciprofloxacin resistance is increasing worldwide and this increase is associated with mutations in quinolone resistance-determining regions (22). These differences in antibiotic resistance rates can be explained by differences in regional antibiotic use intensity and the development of local resistance mechanisms.

Colistin resistance was determined as 3% in our study and 8% in Ukraine (20). Colistin is an antibiotic used in the treatment of digestive system infections caused by Gram-negative bacteria, especially *E. coli* and *Salmonell*a, in poultry (23). The low levels of colistin resistance in both studies can be attributed to the limited use of this antibiotic. Use of colistin in veterinary medicine has been restricted in many countries due to its critical importance for human health and to prevent the spread of antibiotic resistance. In particular, the European Union has restricted the use of colistin in animals and only allows its use in certain cases (23). The spread of colistin resistance poses a serious problem both in veterinary medicine and human health. Therefore, caution should be exercised in the use of colistin and other critically important antibiotics; unnecessary and incorrect use should be avoided, and appropriate measures should be taken to combat antibiotic resistance.

Since antibiotic resistance rates may show regional

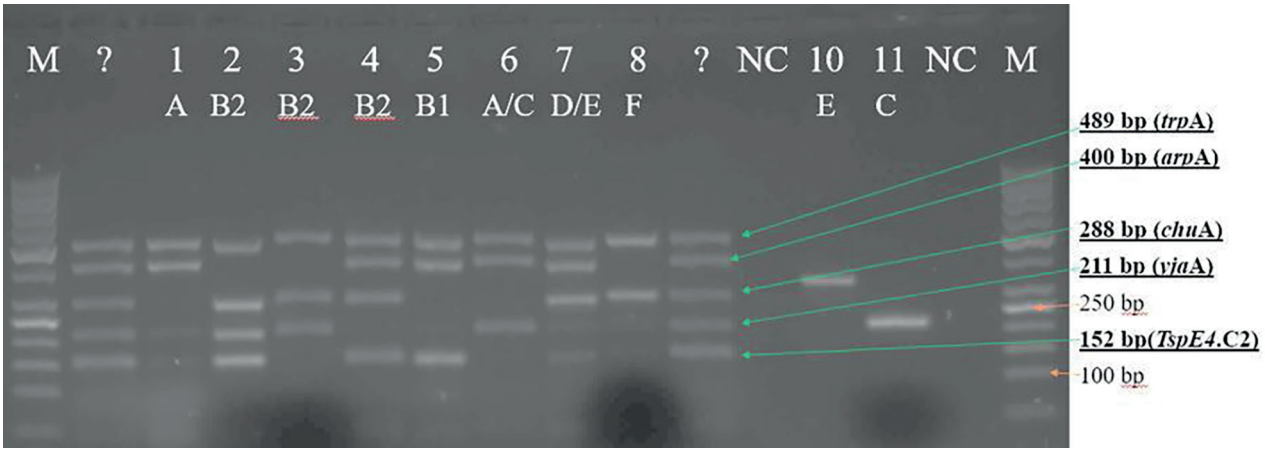

Figure 4. Phylotype profiles of *E. coli* isolates according to the new Clermont phylotyping method. M: Marker (50 bp, Fermentas), 1. Group A (– – – + +), 2. Group B2 (+ + + – +), 3. Group B2 (– + + – +) 4. Group B2 (+ – + + +) 5. Group B1 (+ – – + +), 6. Group A/C (– + – + +), 7. Group D/E (– – + + +), 8. Group F (– – + – +) 9. Unknown Group (?) (Obtained from previous studies) (+ + + + +) (152 bp, 211 bp, 288 bp, 400 bp, 489 bp), NC: DNA free master mix 10. Group C (219 bp) 11. Group E (301 bp) NC: *S.* Enteritidis ATCC 13076 M: Marker (50 bp, Fermentas).

Table 5. Statistical analysis of phylotype distributions

Phylotype	Vaccination		P	χ2
	(+)	(-)		
A (+)	102	5	0.000	125.12
A (-)	78	175		
B1 (+)	49	41	0.394	0.95
B1 (-)	131	139		
B2 (+)	0	19	0.000	20.06
B2 (-)	180	161		
C (+)	0	22	0.000	23.43
C (-)	180	158		
D (+)	0	16	0.000	16.74
D (-)	180	164		
E (+)	21	38	0.022	5.86
E (-)	159	142		
F (+)	8	39	0.000	23.52
F (-)	172	141		

differences, it is of great importance to optimize control strategies applied on farms by taking these resistance profiles into account. In this study, Low resistance rates to amikacin and colistin were observed, consistent with limited exposure to these antibiotics in poultry. On the other hand, limiting the use of antibiotics with high resistance rates, such as tetracyclines, penicillin and quinolones, may be an effective strategy in controlling the development of resistance.

In our study, the detection of multiple antibiotic resistance in 53.8% of *E. coli* isolates in the first five days of broiler life indicates that resistance genes are acquired at an early stage. This situation may be largely due to factors such as vertical transmission from breeder flocks, lack of hygiene in the hatchery environment, contamination of feed and water sources or prophylactic antibiotic use. In addition, the rapid spread of resistance genes via mobile genetic elements may also explain this high rate. This finding emphasizes the need to strengthen biosecurity measures and re-evaluate antibiotic use protocols.

In our study, the effects of commercial attenuated live *E. coli* vaccine on phylogenetic groups were demonstrated. After vaccination, a slight increase in the rate of phylogroup B1 and a significant increase in the rate of phylogroup A were observed, while it was determined that phylogroups B2, C and D with high virulence were completely eliminated. These findings suggest that the commercial vaccine created a strong suppressive effect on all phylogroups except for phylogroup B1. The increase in the prevalence of phylogroup A after vaccination indicates that this group may be more resistant to the effects of the immune system or has become dominant due to environmental and genetic advantages. The lack of significant change in phylogroup B1 may be related to its commensal nature and lower pathogenicity, rather than any specific vaccine-targeted factors. The literature supports that phylogroup B1 mostly carried commensal properties and had a low pathogenicity potential (6,7). This situation

requires a more detailed examination of the selective pressure mechanisms of commercial vaccine on phylogenetic groups.

In a study conducted by Lozica and colleagues in Croatia in 2021, the effects of autovaccine application on the distribution of *E. coli* phylogenetic groups were investigated (24). In the study, a total of 113 *E. coli* strains isolated from clinical necropsies from two different farms were used as material, with commercial vaccine applied to the first flocks and autovaccine applied to the subsequent flocks. Phylogenetic grouping of the isolates was performed using the Clermont multiplex PCR method. In the study, it was determined that the commercial attenuated vaccine had a limited effect on genetically heterogeneous and high virulence potential *E. coli* strains and could not adequately control phylogenetic diversity. The dominance of the B2 phylogroup, especially on farm B, indicated that the commercial vaccine had a low capacity to suppress highly virulent strains in this group. It was observed that autovaccine application caused significant changes in phylogenetic diversity and suppressed many phylotypes, but could not completely eliminate the presence of some phylotypes such as B2. This situation suggests that the effect of autovaccines on targeted phylogenetic groups should be analysed in more detail and shows that strain shifts in phylotype diversity may occur. This study by Lozica *et al.* (2021) highlights the selective effects of autovaccines on phylogenetic groups and the need to optimize the effectiveness of vaccination strategies.

In our study, unlike the study conducted in Croatia in 2021, it was found that the commercial vaccine was more effective in suppressing phylogenetic groups with high virulence potential. In our study, it was found that the commercial vaccine increased the proportion of phylogroups A and B1, while Lozica *et al.* (2021) reported that phylotype diversity in flocks administered commercial vaccine was concentrated in groups A and F. Although Lozica's study included longitudinal data before and after vaccination, differences in study design and strain sources make direct comparison of the effects on phylogenetic changes difficult. This situation reveals the need for more comprehensive data to fully elucidate the changes in phylotype distribution.

Another study conducted by Lozica and colleagues in recent years (25) aimed to evaluate the effects of autovaccine application on *E. coli* genomic diversity, phylogenetic groups and antimicrobial resistance genes. In Lozica's study, commercial vaccine was applied to the first flocks and autovaccine was applied to the following flocks, and 115 *E. coli* strains isolated from clinical cases and target organs from two farms were used as material. The study was carried out using single nucleotide polymorphism analysis and genotypic methods. The results showed that antimicrobial resistance genes decreased over time and that autovaccine application led to genetic homogenization. It was also determined that autovaccine, in addition to its reducing effects on antimicrobial resistance genes, also created a suppression mechanism limiting phylogenetic diversity. The study suggested that more comprehensive studies should be conducted to better understand these findings and to examine the genetic effects in detail.

In both our study and studies conducted in Croatia (24,25), phylogenetic analysis results generally reveal the effects of vaccines, but they exhibited significant differences. In our study, it was determined that the commercial live vaccine completely eliminated high-virulence phylogenetic groups (B2, C, D). In Lozica's 2021 study, it was reported that autovaccine had a suppressive effect on some groups, but could not completely eliminate the B2 group (24). In Lozica's 2022 study, it was determined that autovaccine application provided genetic homogenization, but some highly pathogenic strains (e.g. ST117-F, ST390-B2) continued to show resistance (25). These findings indicate that the effects of the vaccine should be evaluated not only at the level of phylogenetic groups, but also in the context of genetic and phenotypic characteristics of specific strains. Differences in the response to vaccines are directly related to environmental factors, vaccination strategies, and genetic diversity in pathogen populations. This comparison requires extensive research to better understand the effects of different vaccine types on phylogenetic groups, strain diversity and resistance genes.

The findings show that vaccination strategies may have varying effects depending on the pathogenicity potential of different phylogenetic groups and can be considered as an important tool especially in the control of high virulence phylotypes. This situation emphasizes the importance of optimizing vaccination strategies and adapting them according to regional strain profiles.

The Poulvac *E. coli* vaccine used in our study is a live attenuated commercial vaccine that aims to provide protection against a wide range of *E. coli* strains. However, this vaccine does not target specific farm-specific strains and it is known that commercial vaccines generally provide cross-

protection but does not create direct selection pressure for a specific genotype or phylotype (26). Phylotypes A and B1 are generally considered to be low virulence and commensal. As a result of the study, it was determined that vaccination caused phylotype A to become dominant. The immunity provided by the vaccine develops against virulence factors associated with pathogenicity; fimbrial antigens, toxins and capsular antigens. Among the phylotypes, those with higher pathogenicity such as B2, C, D, E and F may contain the factors targeted by the vaccine, which may affect the phylotype distributions.

In conclusion, it was observed that the commercial vaccine suppressed pathogenic phylotypes, leading to the dominance of low pathogenicity strains. This suggests that the vaccine not only reduces the severity of infection but also may cause significant phylotype shifts in the bacterial population. However, it should be noted that phylogenetic changes cannot be explained solely by the effect of the vaccine and that environmental factors, hygiene protocols and antibiotic use policies may also be effective. A more detailed analysis evaluating the effects of these factors is recommended in future studies.

AVAILABILITY OF DATA AND MATERIALS

The corresponding author can provide the datasets of this research upon reasonable request. This manuscript has not been submitted elsewhere for publication, in whole or in part.

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Conflict of Interests

The authors declare that they have no conflicts of interest.

Author Contributions

ES, MKT, ST: Conceptualization, Methodology, Validation, Analysis, Investigation, Writing-Original Draft, Editing. All authors read and approved the final manuscript.

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