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Investigation of the phylogenetic groups, pathotypes, and clonality of extended spectrum β-lactamase encoding *Escherichia coli* strains in the intestine of broilers during rearing periods

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ABSTRACT

The emergence of extended spectrum β-lactamase (ESBL) among pathogenic *Escherichia coli* (*E. coli*) strains could be a threat to poultry and humans. This study aimed to investigate the diversity of ESBL genes among different phylogenetic groups (A, B1, B2, D) and pathotypes of *E. coli* strains (Shiga toxin producing *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*) in the intestines of chicken during the rearing period. PCR was done to characterize the phylogenetic groups, pathotypes, and bla_{TEM}, bla_{SHV}, and bla_{CTX} genes. The homology of *E. coli* strains with ESBL-resistance pattern was analyzed by random amplification of polymorphic DNA-PCR and biotyping methods. In total, 6.3% (28/444) of the strains presented the ESBL phenotype, where carriage of $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}61}$, $bla_{TEM\text{-}116}$, and $bla_{TEM\text{-}1}$ was confirmed after sequencing of the PCR products. Phylogenetic group D was the most prevalent phylogenetic group among ESBL-producing *E. coli* strains (42.85%), followed by group B1 (32.15%), group A (17.85%) and group B2 (7.15%). Two ESBL producing *E. coli* strains belonged to shiga toxin-producing *E. coli* (STEC) and one to atypical enteropathogenic *E. coli* (AEPEC) pathotypes. While identical RAPD-PCR and resistance profiles were detected among ESBL-producing strains in some of the poultry houses during the rearing period, this similarity was not confirmed among different farms. In conclusion, our results showed the presence and gradual dissemination of ESBL producing *E. coli* strains with pathogenic capacity for humans as well as poultry.

Keywords: *Escherichia coli*; poultry; extended spectrum β-lactamase; phylogroups; pathotypes

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Introduction

Escherichia coli is generally considered as a commensal inhabitant of the gastrointestinal tract of humans and animals. However, this bacterium is one of the most frequent causes of intestinal and extra-intestinal diseases (GOLDSTONE et al., 2014; FRANZ et al., 2015). The emergence of antibiotic resistance in *E. coli* strains has become an increasing problem in veterinary and human medicine (VAN DEN BOGAARD et al., 2001; MILES et al., 2006; VINODHINI et al., 2014). Widespread use of antibiotics is the most important factor promoting the emergence, selection, and dissemination of antimicrobial resistance, not only among pathogenic variants of this bacterium, but also among common members of the intestinal microbiota in animals and humans.

The emergence of new antibacterial resistance phenotypes in *E. coli* strains, especially against broad spectrum antibiotics, is a leading cause of therapeutic failures (SIMÕES et al., 2012; YADAV et al., 2015). Extended spectrum ß-lactamase (ESBL) encoding genes are mainly carried on plasmids, which enables their easy transmission between different strains along the food chain, and poses a risk factor for dissemination of the relevant resistance genes in the community. Nowadays, increasing attention is being paid to the importance of ESBL producing *E. coli* strains in the intestinal tract of broilers (GIRLICH et al., 2007; MIRZAEE et al., 2009; DAHMS et al., 2015). Enrichment of highly virulent *E. coli* strains in the intestine of poultry upon exposure to antibiotics could occur, which is mainly mediated by co-carriage of these resistance genes with virulence factors. This effect leads to the increased disease potential of *E. coli* populations.

Molecular characterization and population structure study of *E. coli* strains could provide valuable data to track and control diseases originating from the pathogenic isolates in specific hosts. While cattle and ruminants are the main reservoirs of *E. coli* pathotypes for humans, including mainly ETEC (Enterotoxigenic *E. coli*), EPEC (Enteropathogenic *E. coli*), EIEC (Enteroinvasive *E. coli*), EHEC (Enterohemorrhagic *E. coli*), and EAEC (Enteroaggregative *E. coli*), the role of poultry in transmission of these pathotypes and related ESBL-encoding genes is still not clear (SIMÕES et al., 2012; GIRARDINI et al., 2012; TRKOV et al., 2014). The relatedness of these pathotypes to common phylogroups of *E. coli* (A, B1, B2, and D) (DERAKHSHANDEH et al., 2013; JAFARI et al., 2016), and the ability to acquire the transferrable plasmids carrying ESBLs genes is also unknown (SALEHI et al., 2008; KUMAR et al., 2015). Given the widespread emergence of antibacterial resistance, especially ESBL producing *E. coli* strains, and the global threat of dissemination of resistant bacteria in humans and animals, there is a need to study the probable reservoirs of these highly virulent strains in food producing animals. To show these relationships, continuous monitoring of changes, genetic background, resistance phenotypes, and the virulence entity of *E. coli* strains over different time periods is necessary.

This study aimed to investigate the initial intestinal colonization and dissemination of related pathotypes and phylogroups of ESBL-producing *E. coli* strains in broilers in different poultry houses during rearing periods.

Materials and methods

Samples and bacterial isolates. To evaluate alterations in the population structure of the colonizing intestinal *E. coli* isolates during the rearing period, sampling was performed in two separate phases of growth. Accordingly, the samples were collected from birds less than a week old (phase I samples), and from birds a few days before slaughter (days of 40-42 of the rearing period) (phase II samples). Five hundred cloacal swabs were taken from broilers of 5 different poultry flocks in a nearby geographical region in Tehran province. The swab samples were transported to the laboratory in Cary-Blair medium (Merck, Germany). Then, the swabs were inoculated onto MacConkey agar medium (Merck, Germany). The *E. coli* isolates were identified by conventional microbiological methods and biochemical testing (LUCE, 2010). Out of the 444 *E. coli* strains detected, 174 strains (39.2%) relating to phase I and 270 strains (60.8%) relating to phase II were included in the study.

MIC detection in ESBLs producing E. coli strains. In a previous study, just 6.5% of the broilers′ strains were determined to be ESBL$^+$ -*E. coli* strains (DOREGIRAEE et al., 2017). Therefore, the minimum inhibitory concentration (MIC) value of ceftazidime was measured for the strains that showed ESBL resistance phenotype. The agar dilution method, according to the CLSI guidelines, was used to show MIC ranges. Briefly, ceftazidime powder (Sigma Aldrich, USA) solution was added to the molten Mueller–Hinton agar medium (Merck, Germany) to provide two-fold dilutions, ranging from 4.0 µg/ml to 128 µg/ml. Bacterial suspensions were applied to the agar plates, and the results were read following incubation at 35°C for 18–20 h. The MIC breakpoint used for the susceptibility testing was ≤4 µg/ml. *E. coli* ATCC 25922 was used as the reference strain in all experiments (WAYNE, 2014).

Plasmid mediated transmission of ESBLs encoding genes. To show the presence of ESBL genes on the plasmids of the strains with the ESBLs resistance phenotype, the plasmid extracts were introduced to competent *E. coli* DH5α (prepared at the Food Safety Research Center, University of Shahid Beheshti Medical Sciences). The plasmid extracts were prepared using the alkaline lysis method (SAMBROOK and RUSSELL, 2001). 15 µL of plasmid extract was mixed with100 µL of *E. coli* DH5α and incubated for 10 minutes on ice. Then heat-shock of the mixture at 42ºC was performed for 2 min. 1 ml of Luria-Bertani (LB) broth medium was added and incubated at 37ºC with a slow shake (100 rpm) for 60 min. 100 µL of the transformants were transferred to Luria-Bertani (LB) agar medium, supplemented with amoxicillin (50 µg/ml) and incubated overnight at 37ºC. The presence of the *bla* genes was detected by PCR, and the MIC of ceftazidime was measured for the transformants, as described before (DOREGIRAEE et al., 2017).

Diversity of E. coli pathotypes among ESBLs producing strains. All the ESBL producing *E. coli* strains were evaluated to the determine common pathotypes that are involved in intestinal diseases in humans and animals. PCR assays for detection of STEC (stx_1 and stx_2 genes), ETEC (*st* and *lt* genes), EIEC (*ipaH* and *eae* gene), typical EPEC (*bfp* and *hly* genes), and EAEC (*aggR*) were performed using the primer pairs listed in Table 1. The PCR assays were carried out on the summation of all volumes that comprised 25 µl (11 µl of 2x Hot Star Taq Master Mix, 30 ng of the DNA template, 1 µl of each primer (20 pmol) using the Hot Star Taq Master Mix kit (SinaClon, Iran). DNA amplification was carried out in a thermocycler (Eppendorf, Hamburg, Germany) with an initial denaturation step at 95°C for 10 minutes, 35 amplification cycles for 45 seconds each at 95°C; 45 seconds at different temperatures for the various genes (Table 1); and 50 seconds at 72°C, followed by an additional extension step of 7 minutes at 72°C. The amplified products were visualized using gel electrophoresis on 1% gel agarose containing 1x GelRed DNA stain (Biotium, Inc., USA).

Phylogenetic group analysis. For determination of major *E. coli* phylogenetic groups (A, B1, B2 and D), carriage of the *chuA* and *yjaA* genes and TspE4.C2 fragments were examined by the multiplex PCR-based method described by CLERMONT et al. (2000). Nucleotide sequences of the primers used in this study are shown in Table 1. The PCR mixture was prepared as described above, and amplification of the targeted genes was done as follows: one cycle of initial denaturation for 5 minutes at 94°C, followed by 30 cycles of 30 seconds denaturation at 94°C, annealing for 30 seconds at 55°C, and extension for 30 seconds at 72°C. The final 7 minute extension was done at 72°C for one cycle.

Screening of O157 E. coli strains with the ESBL phenotype. Isolation of shiga toxigenic *E. coli* (STEC) strains related to EHEC variants was done by culture of the ESBL producing strains on Sorbitol-MacConkey agar (SMAC). The non-sorbitol fermenting isolates were tested for O157 antigen by the latex agglutination method (B.I.R.D. Baharafshan, Iran) (FARMER and DAVIS, 1985).

RAPD-PCR Analysis. The genomic relatedness of *E. coli* isolates with resistance patterns linked to the ESBL phenotype was analyzed using RAPD-PCR. The reaction was done using primer 1283 (5'-GCGATCCCCA-3'), as described before (JOHNSON et al., 2003). The RAPD-PCR was

done in a 25 µl volume containing 2.5 µl of 10X PCR buffer solution, 10 mM deoxynucleotide triphosphate (dNTP), of 50 mM $MgCl_2$, 10 pmol primer, 0.2 units of Taq DNA polymerase, and a 30-40 ng DNA template. Amplification was performed using a thermal cycler (Mycycler, Bio Rad, USA) with a temperature program consisting of 4 cycles of denaturation at 94°C for 4 minutes, annealing at 36°C for 4 minutes and polymerization at 72°C for 4 minutes, followed by 40 amplification cycles for 30 seconds each at 95°C, 1 minute at 36°C, and 2 minutes at 72°C. Final elongation was at 72°C for 10 minutes. The amplified products were detected using electrophoresis on 2% gel agarose containing 1x GelRed DNA stain (Biotium, Inc., USA). The bands from the gels were visualized by gel documentation software (Quantity One, Bio-Rad, USA) and a dendrogram was constructed using UPGMA software available online (http://genomes.urv.cat/ UPGMA).

Table 1. Oligonucleotide primers used in this study

Gene name		Primer sequence (5′-3′)	Product size (bp)	Annealing (ºC)
STEC	*stx*$_1$	Fw-GAAGAGTCCGTGGGATTACG Rv-AGCGATGCAGCTATTAATA	130	30
	stx$_2$	Fw-GGATGCATCTCTGGTCATTG Rv-CTTCGGTATCCTATTCCCGG	478	30
ETEC	*st*	Fw-TGTCTTTTTCACCTTTCGCTC Rv-CGGTACAAGCAGGATTACAACAC	171	30
	lt	Fw-GAACAGGAGGTTTCTGCGTTAGGTG Rv-CTTTCAATGGCTTTTTTTGGGAGTC	655	30
EIEC	*ipaH*	Fw-CCTTTTCCGCGTTCCTTGA Rv-CGGAATCCGGAGGTATTGC	426	53
	eae	Fw-TCAATGCAGTTCCGTTATCAGTT Rv-GTAAAGTCCGTTACCCCAACCTG	482	54
Typical EPEC	*bfp*	Fw-CACCGTTACCGCAGGTGTGA Rv-GTTGCCGCTTCAGCAGGAGT	450	63
	ehxA, hly	Fw-AGCTGCAAGTGCGGGTCTG Rv-TACGGGTTATGCCTGCAAGTTCAC	569	55
EAEC	*aggR*	Fw-CTAATTGTACAATCGATGTA Rv-AGAGTCCATCTCTTTGATAAG	457	48
Phylogroup	*chuA*	Fw-GACGAACCAACGGTCAGGAT Rv-TGCCGCCAGTACCAAAGACA	279	55
	yjaA	Fw-TGAAGTGTCAGGAGACGCCTG Rv-ATGGAGAATGCGTTCCTCAAC	211	55
	TspE4.C2	Fw-GAGTAATGTCGGGGCATTCA Rv-CGCGCCAACAAAGTATTACG	152	55
RAPD-PCR		GCGATCCCCA		36

Supplementary Table 1. Minimum inhibitory concentrations of ceftazidime among extended spectrum β-lactamase producing *E. coli* isolates in poultry

MICs of ceftazidime	4 µg/ml N (%)	8 µg/ml N (%)	16 µg/ml N (%)	32 µg/ml N (%)	64 µg/ml N (%)	124 µg/ml N (%)
ESBL producing *E. coli* (N=28)	0 (0)	0 (0)	5 (17.9)	18 (64.2)	5 (17.9)	0 (0)

N=number

Table 2. Prevalence of multiple drug resistant *E. coli* strains with ESBL phenotype among the resistant strains to 3rd generation cephalosporins and cephamycins

MDR patterns [a]	Frequency (%)										Total	ESBL phenotype[d]
	A		B		C		D		E			
	I[b]	II[c]	I	II	I	II	I	II	I	II		
CMY	0	0	0	0	0	8 (19%)	0	0	33 (49.2)	52 (54.2)	93 (22.3) 42 (9.7)	0
CMY+E-CEPH	0	4 (7.9)	13 (92.8)	7 (21.2)	2 (10.5)	8 (19%)	3 (14.3)	5 (15.1)	0	0		28
Total	38 (80.4)	50 (98.2)	14 (100)	30 (84)	19 (100)	38 (100)	22 (77.7)	26 (99.8)	65 (91.1)	97 (95.6)	399 (91.9)	28 (6.3)
ESBL phenotype	**0 (0)**	**3 (5.6)**	**2 (14.3)**	**6 (18.7)**	**0 (0)**	**4 (9.5)**	**7 (33.3)**	**5 (15.1)**	**0 (0)**	**1 (1.06)**	**28 (6.3)**	

[a]MDR: multiple drug resistance phenotype; CMY: Cephamycins (Cefoxitin); E-CEPH: extended-spectrum cephalosporins (Cefotaxime, Ceftazidime, Ceftriaxone);

[b] I: Phase I

[c] II: Phase II

[d] ESBL phenotype: *E. coli* strains with a resistance phenotype associated with extended spectrum β-lactamase-producing bacteria

Results

Distribution of the ESBL phenotype among the strains with MDR patterns. Out of 399 *E. coli* strains (399 /444, 92.3%) that were resistant to 3 classes of antibiotics (MDR strains), resistance to cephamycins (Cefoxitin) and 3rd generation cephalosporins (cefotaxime, ceftazidime, ceftriaxone) was detected in 21.8% (94/430) and 9.7% (42/430) of them, respectively. The prevalence of ESBL producing strains was 6.5% (28/430) among the strains with a MDR phenotype. 9/430 (2.1%) were isolated from the phase I, and 19/430 (4.4%) from the phase II birds (Table 2). The ESBL producing *E. coli* strains showed MIC values in a range of 16-64 µg/ml for ceftazidime (MIC_{50}, 32 µg/ml and MIC_{90}, 64 µg/ml) (Supplementary Table 1).

Phylogenetic group analysis. The results of the phylogenetic analysis revealed that the ESBL producing *E. coli* isolates were mostly linked to phylogenetic group D (12 isolates, 42.85%), followed by group B1 (9 isolates, 32.15%), group A (5 isolates, 17.85%) and group B2 (2 isolates, 7.15%). The distribution of the phylogenetic groups in ESBL producing strains of *E. coli* and the changes in their patterns during rearing periods are shown in Table 3.

Frequency of E. coli pathotypes among the ESBL encoding isolates. PCR assays for *E. coli* pathotypes revealed the presence of at least one virulence gene, including stx_2, *eae* and *hly* in 26.42% of the isolates (6/28). Among the STEC isolates (7.1%, 2/28), one of the isolates carried both stx_2 and *ehxA* genes, whereas the other isolate was positive for just the stx_2 gene. Furthermore, in the absence of *stx* and *bfp* genes, *eae* was detected in one isolate (atypical EPEC) that belonged to the B1 phylogenetic group. The results showed the sole carriage of the *exhA* gene in three isolates. The results of the agglutination assay for detection of *E. coli* O157:H7 strains showed that the two STEC isolates belonged to the *E. coli* O157:$H7^{(-)}$ serotype. The two strains were associated with phylogenetic groups D and B1.

RAPD-PCR analysis. To show the relatedness of *E. coli* strains among the isolates originating from the same farm and those originating from different farms, RAPD-PCR was performed. The analysis showed 71 RAPD types among seventy-nine *E. coli* strains that showed similar resistance phenotypes. Identical RAPD patterns were detected in 12 strains (15.2%), while different and closely related patterns were characterized among 24 (30.4%) and 43 (54.4%) isolates, respectively. These relationships are depicted in a phylogenetic dendrogram constructed by the un-weighted paired group method of arithmetic average (UPGMA) (Fig. 1). Our findings did not show any similarity among the strains isolated from the five different poultry farms. The identical and related RAPD

Table 3. Phylogenetic group analysis of ESBL producing *E. coli* strains in five poultry houses

Phylogenetic group	Sampling phases		Poultry houses (phase I and II)										Total (%)
	Phase I (%)	Phase II (%)	A		B		C		D		E		
			I	II	I	II	I	II	I	II	I	II	
A	3 (60)	2 (40)	0	0	3	1	0	1	0	0	0	0	5 (17.85)
B1	2 (22)	7 (78)	0	1		3	0	1	2	1	0	1	9 (32.15)
B2	0 (0)	2 (100)	0	2		0	0	0	0	0	0	0	2 (7.15)
D	6 (50)	6 (50)	0	0		1	0	2	6	3	0	0	12 (42.85)

Phase I: chickens less than a week old
Phase II: broilers a few days before slaughter

patterns were associated with poultry house D (more importantly 2 $bla_{CTX\text{-}M\text{-}1}$-producing strains from phase I and 2 $bla_{CTX\text{-}M\text{-}1}$-producing strains from phase II of the sampling), and poultry house A (mainly including three isolates from the broilers (1 strain from phase I and 2 strains from phase II) (Fig. 1).

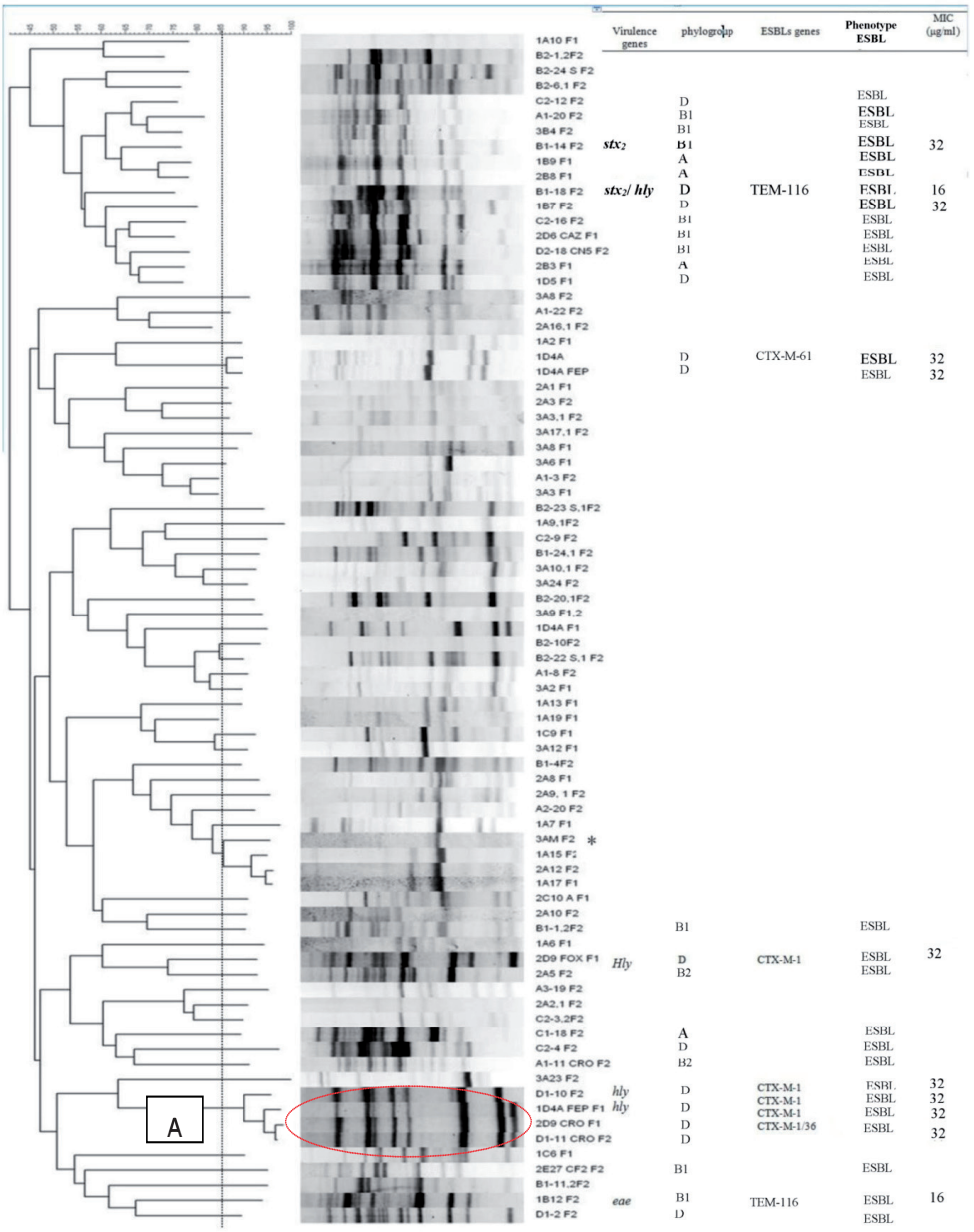

Fig. 1. Phylogenetic analysis of *E. coli* isolates from poultry cloaca using RAPD-PCR analysis and their divergence as phylogroups, resistance to antibiotics, and resistance gene markers during the rearing period

Vertical dashed line: 85% cut-off line. TE: Tetracycline, AMC: Ampicillin,C: Chloramphenicol, GM: Gentamicin, CN: Cephalexin, CF: Cephalotin, FOX: Cefoxitin, CRO: Ceftriaxone. Cluster A: *E. coli* strains with the same MIC value, same RAPD pattern, identical phylogroup, isolated from the same farm at the two time points of sampling. F, phase of sampling; A-D, phylogroups A-D; hly, hemolysin; eae, effacement and attachment gene locus; stx, shiga toxin gene.

Discussion

E. coli is a member of the intestinal microbiota in animals and humans. This bacterium is the most common enteric organism causing various intestinal and extra-intestinal infections (DERAKHSHANDEH et al., 2013; TRKOV et al., 2014). Colonization of pathogenic *E. coli* strains with resistance to broad spectrum antibiotics in the intestinal tract of broilers could be a threat to poultry and humans. The antimicrobial resistance of these strains in the intestinal tract could limit treatment options for the intestinal and extraintestinal infections causes by them (MIRZAEE et al., 2009; ZHANG et al., 2016).

In the present study, we studied the ESBL producing strains from our previous study (DOREGIRAEI et al., 2017). Despite the low incidence of ESBL producing *E. coli* strains (6.5%) in our previous study, an alarming increase in the incidence of this phenotype has been reported globally. In the studies performed in the recent years, the prevalence of ESBL-producing *E. coli* was detected in 25.9% (TANSAWAI et al., 2019), 32.2% (AWORH et al., 2020) and 46.7% (BADR et al., 2022) of poultry samples, which shows the importance of poultry as the source of the ESBL phenotype in humans. BENINATI et al. (2015) showed that 100 percent of *E. coli* isolates from chicken products had the ability to produce ESBLs. In another study, the frequency of ESBL-producing *E. coli* strains from healthy poultry was reported in 10.7% of the isolates. They stated that food animals can be a possible reservoir for ESBL genes in transferable plasmids (GIRLICH et al., 2007). LEVERSTEIN-VAN HALL et al. (2011) found that the presence of ESBL producing bacteria in the gastrointestinal tract of food animals may contribute to the increased incidence of infections caused by these bacteria in human populations.

The results of this study showed a higher frequency of ESBL producing *E. coli* isolates in phase II of the sampling period (64.3%), which could be mediated by selective pressure by the antibiotics used during the rearing period. Detection of ESBL producing *E. coli* from chickens less than one week of age demonstrates the risk of introducing ESBL producing *E. coli* to the broiler production chain when buying positive chicks for restocking grandparent or breeder flocks. The results of other studies also reinforce the risk of contamination of grandparent flocks with ESBL producing *E. coli* (MACHADO et al., 2008; LAUBE al., 2013).

Diarrheagenic *E. coli* pathotypes, including ETEC, EAEC, EIEC, EPEC, EHEC and DAEC, are recognized as an important cause of intestinal infection in humans and animals. However, it is worth mentioning that intestinal *E. coli* infections are rare in poultry (NOLAN et al., 2020). On the basis of previous phylogenetic analysis in mammals in Iran, the great majority of diarrheagenic and commensal *E. coli* strains were distributed among groups A, B1 and D (GHANBARPOUR et al., 2010; ALIZADE et al., 2014). In another study, B1 was the main phylogroup in poultry farms in Iran (STAJI et al., 2018). Our analysis revealed that the most prevalent phylogenetic group among the ESBL producing *E. coli* isolates was phylogenetic group D (42.85%), which are generally linked to the strains responsible for human extra-intestinal infections (CORTES et al., 2010). Phylogenetic groups B1 (32.15%), A (17.85%), and B2 (7.15%) composed the other groups characterized among these isolates. These findings are consistent with other studies (EWERS et al., 2009; REICH et al., 2013). In a study by REICH et al. (2013), similar proportions of phylogenetic groups in healthy broiler chickens were shown (A (31.5%), B1 (20.2%), B2 (13.5%), and D (34.8%)). Several studies have shown that some phylogenetic groups, mainly B2, and to a lesser extent phylogenetic group D, are of public health concern since they contain strains of higher pathogenic character. Furthermore, strains belonging to phylogenetic group B2 can be found in diseased and healthy poultry, and could have zoonotic potential (EWERS et al., 2009; REICH et al., 2013). Research data showed an association between intestinal colonization with *E. coli* strains belonging to phylogenetic group B2 and the occurrence of inflammatory bowel disease (IBD) in humans, which demonstrates the importance of further studies in this regard (SOBIESZCZANSKA et al., 2011).

We found only three virulence genes (stx_2, *eae* and *hly*) in the ESBL-producing *E. coli* isolates. One

isolate was positive for both the stx_2 and *hly* genes, whereas the other isolate was only positive for the stx_2 gene. Furthermore, the *eae* gene was detected in one ESBL-producing *E. coli* isolate. Carriage of *hly* as the sole virulence factor associated with general pathotypes of *E. coli* was detected in three isolates. According to these results, two ESBL-producing isolates belonged to the STEC pathotype, while one isolate was identified as an atypical EPEC pathotype. The results demonstrated that two *E. coli* O157 non-H7 strains belonged to phylogenetic groups D and B1. Furthermore, the atypical EPEC strain detected in this study was associated with group B1. Only a few studies have shown the incidence of the STEC pathotype in *E. coli* isolates from poultry (COBELJIĆ et al., 2005; BOHAYCHUK et al., 2009; FAROOQ et al., 2009). Our study is consistent with the observations of Krause et al. (2005) which showed that the majority of animal isolates were atypical EPEC. It has already been proven that typical EPEC is rarely isolated from animals, while atypical EPEC strains could be isolated from both humans and animals (AKTAN et al., 2004).

The molecular typing results showed that 79 isolates could be differentiated to 71 RAPD types, which demonstrates the high genetic diversity of the studied strains. Out of the 79 *E. coli* isolates, 12 (15.2%) strains had identical RAPD patterns. The results indicated 24 (30.4%) and 43 (54.4%) strains as different and closely related, respectively. This study did not show any clonal spread between the *E. coli* strains isolated from different poultry houses. Clonality was only observed between four strains on one poultry farm, which were isolated in both phases of the sampling.

Our findings reinforce the risk of introducing ESBL producing *E. coli* into the broiler production chain. The study established the presence of STEC and atypical EPEC pathotypes with ESBL phenotypes and the presence of ESBL-encoding genes among the E. coli isolates on poultry farms in Iran. In addition, the association of these strains to phylogenetic groups associated with human diseases could be considered as a public health concern.

Author's contributions

Bahar Nayeri Fasaei and Masoud Alebouyeh contributed equally to this study

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical approval

Ethical approval was obtained from the Ethics Committee at the Faculty of Veterinary Medicine, University of Tehran (Code 615-T), Tehran, Iran.

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DOREGIRAEE, F., B. N. FASAEI, M. ALEBOUYEH, S. CHARKHKAR, E. TAJEDDIN, A. GHODDUSI: Istraživanje filogenetskih skupina, patotipova i klonalnosti β-laktamaze proširenog spektra koja kodira sojeve *Escherichia coli* u crijevima tovnih pilića tijekom različitih razdoblja uzgoja. Vet. arhiv 94, 129-140, 2024.

SAŽETAK

Pojava β-laktamaze proširenog spectra (ESBL) među patogenim sojevima bakterije *Escherichia coli* (*E. coli*) mogla bi biti prijetnja i za perad i za ljude. Cilj je rada bio istražiti raznolikost ESBL gena među različitim filogenetskim skupinama (A, B1, B2 i D) i patotipovima sojeva *E. coli* (*E. coli* koja proizvodi *shiga*-toksin, enteroinvazivna *E. coli*, enteropatogena *E. coli*, enterotoksigena *E. coli*) u crijevima pilića tijekom različitih razdoblja uzgoja. Za karakterizaciju filogenetskih skupina, patotipova i gena bla_{TEM}, bla_{SHV} i bla_{CTX} učinjen je PCR. Homolognost sojeva *E. coli* s ESBL-rezistentnim uzorkom analizirana je metodom nasumičnog umnažanja polimorfne DNA – PCR i metodama biotipizacije. Ukupno je 6,3% (28/444) sojeva predstavljalo fenotip ESBL, pri čemu je prijenos $bla_{CTX\text{-}M\text{-}1}$, $bla_{CTX\text{-}M\text{-}61}$, $bla_{TEM\text{-}116}$ i $bla_{TEM\text{-}1}$ potvrđen nakon sekvenciranja PCR produkata. Među sojevima *E. coli* koji proizvode ESBL najzastupljenija je bila filogenetska skupina D (42,85%), zatim skupina B1 (32,15%), skupina A (17,85%) i skupina B2 (7,15%). Dva su soja *E. coli* koja proizvode ESBL pripadala *E. coli* koja proizvodi *shiga*-toksin (STEC), a jedan atipičnim enteropatogenim patotipovima *E. coli* (AEPEC). Premda su u nekim peradarnicima među sojevima koji proizvode ESBL tijekom uzgoja otkriveni identični RAPD-PCR i profili rezistencije, ova podudarnost nije potvrđena među različitim farmama. Zaključno, naši su rezultati pokazali prisutnost i postupno širenje sojeva *E. coli* koji proizvode ESBL i koji imaju patogeni kapacitet i za ljude i za perad.

Ključne riječi: *Escherichia coli*; perad; β-laktamaze proširenog spectra; filogenetska skupina; patotipovi