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Phylogenetic typing and detection of extended-spectrum β-lactamases in *Escherichia coli* isolates from broiler chickens in Ahvaz, Iran

Ramezan Ali Jafari[1]*, Hossein Motamedi[2], Elham Maleki[3], Reza Ghanbarpour[4], Mansoor Mayahi[1]

[1] Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; [2] Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran; [3] Post Graduate Student, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran; [4] Department of Microbiology, Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman, Kerman, Iran.

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Abstract

This study was conducted to reveal the phylogenetic background, to detect the genes encoding TEM, SHV and CTX-M-15 extended-spectrum β-lactamases (ESBL), and to analyze their distribution in phylo-groups of 150 *Escherichia coli* isolates from broiler chickens in Ahvaz (Southwest of Iran). Seventy- five cloacal swabs from healthy birds (fecal isolates), and 75 heart blood samples from birds with *colibacillosis* (septicemic isolates) were obtained. All isolates were phylotyped and screened for ESBL genes by polymerase chain reaction (PCR). The fecal isolates belonged to four main phylo-groups, including 41 isolates (54.67%) to A, 9 (12.00%) to B1, 5 (6.67%) to B2, and 20 (26.67%) to D. Of septicemic isolates, 37 isolates (49.33%) were classified as phylotype A, 5 (6.67%) as B1, 10 (13.33%) as B2, and 23 (30.67%) as D. In molecular analysis, a total of 72 isolates (35 fecal and 37 septicemic) were identified to harbor ESBL genes, which were distributed in phylo-groups A, B1, B2, and D. Regardless of the type of isolate, $bla_{\text{CTX-M-15}}$ gene was the most common genotype, followed by bla_{TEM} and bla_{SHV} genes. This study suggests that broiler chickens in Iran are infected to ESBL genes- harboring *Escherichia coli* strains which may be spread to the food chain through fecal contamination of carcasses during slaughtering.

تعیین گروه فیلوژنی و ردیابی بتالاکتامازهای وسیع الطیف در جدایه های *اشریشیا کولی* از ماکیان گوشتی در اهواز، ایران

چکیده

این مطالعه با هدف تعیین گروه فیلوژنی، برای شناسایی ژن‌های مولد بتالاکتامازهای وسیع‌الطیف (ESBL) TEM، SHV و CTX-M-15، و نیز بررسی توزیع آن‌ها در گروه‌های فیلوژنی در ۱۵۰ جدایه *اشریشیا کولی* جدا شده از گله های گوشتی در اهواز (جنوب غرب ایران) انجام گرفت. هفتاد و پنج سواب کلواک از پرندگان سالم (جدایه‌های مدفوعی) و ۷۵ نمونه خون قلب از پرندگان دارای کلی‌باسیلوز (جدایه‌های سپتی‌سمیک) گرفته شدند. تمامی جدایه‌ها از نظر گروه فیلوژنی و حضور ژن‌های ESBL به وسیله واکنش زنجیره‌ای پلی‌مراز بررسی گردیدند. جدایه‌های مدفوعی در چهار گروه فیلوژنی: A با ۴۱ جدایه (۵۴/۶۷ درصد)، B1 با ۹ جدایه (۱۲/۰۰ درصد)، B2 با ۵ جدایه (۶/۶۷ درصد) و D با ۲۰ جدایه (۲۶/۶۷ درصد) قرار داشتند. از جدایه‌های سپتی‌سمیک، ۳۷ جدایه (۴۹/۳۳ درصد) در گروه A، ۵ جدایه (۶/۶۷ درصد) در B1، ۱۰ جدایه (۱۳/۳۳ درصد) در B2 و ۲۳ جدایه (۳۰/۶۷ درصد) در D طبقه بندی شدند. در بررسی مولکولی، در مجموع ۷۲ جدایه (۳۵ مدفوعی و ۳۷ سپتی‌سمیک) حامل ژن‌های ESBL شناسایی شدند که در چهار گروه فیلوژنی A، B1، B2 و D قرار داشتند. صرف نظر از نوع جدایه، ژن $bla_{\text{CTX-M-15}}$ بالاترین فراوانی را داشت و پس از آن ژن‌های bla_{TEM} و bla_{SHV} در مراتب بعدی بودند. این مطالعه نشان می دهد جوجه‌های گوشتی در ایران به *اشریشیا کولی* حامل ژن‌های ESBL آلوده هستند که ممکن است در فرآیند کشتار از طریق آلودگی مدفوعی لاشه ها به زنجیره غذایی راه یابند.

واژه های کلیدی: *اشریشیا کولی*، بتالاکتاماز، فیلوژنی، ماکیان

***Correspondence:**

Ramezan Ali Jafari. DVM, DVSc
Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
E-mail: jafari.ramezanali@scu.ac.ir

Introduction

Escherichia coli is a normal inhabitant of gastro-intestinal tract of humans and animals.[1] Phylogenetic studies have indicated that the great majority of *E. coli* strains can be classified into one of the four phylo-groups (A, B1, B2, and D) according to the combination of the three genetic markers: *chuA*, *yjaA*, and DNA fragment *TspE4.C2* as previously described by Clermont *et al*.[2] The diversity of genetic background among the phylo-groups apparently influences their antibiotic-resistance profile and growth rate,[3] prevalence of virulence factors,[4] and their ability to establish a population.[5] Johnson *et al.* found that the strains in phylo-groups B2 and D carried more virulence factors than those in groups A and B1.[4] Gordon and Cowling observed that the relative prevalence of phylogenetic groups among host individuals depended on the host habitat, host diet, typical body temperature, gut morphology and geographical factor. They also suggested that the competitive interactions among strains within a host may play a role as well.[5]

In poultry, some intestinal strains referred to as avian pathogenic *E. coli* (APEC) may secondarily cause a local or systemic extra-intestinal infection which is responsible for significant losses and antimicrobial treatment costs.[6,7] In the past few years, both the incidence and severity of infection with APEC isolates have increased rapidly, and it is likely to become an even greater problem in poultry industry.[8] Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis.[9] β-lactam antibiotics are one, if not the most important, group of antimicrobial agents used in medicine and veterinary medicine. Resistance to β-lactam antibiotics in bacterial agents is mostly mediated by β-lactamases which hydrolyze the β-lactam ring and thus inactivate the antibiotic.[1] Up to now, more than 400 β-lactamases have been reported, and new β-lactamases continue to emerge worldwide.[10] β-lactamases are classified on the basis of their primary structures into four molecular classes (A through D).[11] Class B and C β-lactamases have a broader spectrum of activity, but are nearly always encoded by chromosomal genes, and hence are confined to particular bacterial species.[12] Enzymes TEM, SHV and CTX-M belong to class A, being the predominant plasmid-mediated extended-spectrum β-lactamases (ESBLs) in Gram-negative bacteria, and can be easily transferred between and within bacterial species.[12,13] In some cases, the presence of ESBLs in *Enterobacteriaceae* bacteria has been accompanied with resistance to non-β-lactam antibiotics such as fluro-quinolones and aminoglycosides.[14] At slaughter-houses, fecal contamination of poultry carcasses could spread the resistant bacteria to the food chain, and thus transfer resistance genes to human pathogenic bacteria- which are serious menaces to public health.[15] To our knowledge, data correlating β-lactamases in commensal and pathogenic *E. coli* strains from animal origin in Iran are limited. Therefore, this study was carried out to determine the phylogenetic background, to establish the prevalence of ESBL genes encoding TEM, SHV and CTX-M-15, and to analyze the phylogenetic group distribution of the resistance genes in *E. coli* isolates recovered from broiler chickens in Ahvaz (Southwest of Iran).

Materials and Methods

Sample collection. Between April 2013 and March 2014, a total of 15 broiler chicken farms located around Ahvaz city (Southwest of Iran) were sampled. The birds were aged between 21 and 42 days, and had not received any antibiotic medication at least one week before sampling. From each farm, five cloacal swabs from healthy birds (totally 75 fecal isolates) and five heart blood samples from birds with overt lesions of *colibacillosis* (totally 75 septicemic isolates) were obtained.

Bacterial examination. Samples were plated onto MacConkey agar (Merck, Darmstadt, Germany), and incubated overnight at 37 ˚C. One of the pink colonies from each plate was picked, streaked onto eosin methylene blue agar (Merck) plate, and incubated overnight at 37 ˚C. Then, one colony with dark-blue center and green metallic sheen was selected from the solid medium, and identified as *E. coli* by using a panel of biochemical tests that included gas production and sugar fermentation reaction on triple sugar iron agar, indole production, motility, citrate fermentation, urease production, lysine decarboxylation, and methyl red Voges-Proskauer.[16] Finally, all isolates were stored in tryptic soy broth (Merck) with 30.00% sterile glycerol (Merck) at – 70 ˚C.

Antimicrobial susceptibility testing. The anti-microbial susceptibility of all *Escherichia coli* isolates was performed according to Kirby-Bauer disc diffusion method. Clinical and Laboratory Standards Institute (CLSI) guidelines were followed for inoculum standardization, medium, incubation condition, and internal quality control organism (*E. coli* ATCC 25922). Briefly, after adjusted in phosphate-buffered saline to 0.5 McFarland standard, the inoculum was triple streaked (a 60˚ rotation of the round Petridish within streaks) on Mueller-Hinton agar (Oxoid, Basingstoke, UK) plate. Then, antimicrobial discs (Padtan-Teb Co., Tehran, Iran) were placed manually onto the medium by means of a sterile forceps. A combination of cefotaxime (30 µg) and ceftazidime (30 µg) with and without clavulanic acid (10 µg) was used. If the diameter of the inhibition zone in the double disc was at least five millimeters more than that in a single disc, the isolate was considered to be an ESBL producer.[17]

DNA extraction. All *E. coli* isolates were cultured onto nutrient agar (Merck) plates for 20 hr at 37 ˚C. Then, five colonies were transferred to sterile distilled water in a

sterile Eppendorf tube (Greiner, Austria), and boiled to prepare template DNA for PCR. The templates were stored at – 20 ˚C.[18]

Phylogenetic group determination. According to Clermont *et al.,* the phylogenetic group of each strain was determined by multiplex PCR of the genes *chuA* and *yjaA* and the DNA fragment TspE4.C2.[2] The amplification products were separated in 1% agarose gel (Cinagen, Tehran, Iran). After electrophoresis, the gel was photographed under ultraviolet light, and the strains were allocated to phylo-genetic groups A (*chuA*-, *TspE4.C2*-), B1 (*chuA*-, *TspE4.C2*+), B2 (*chuA*+, *yjaA*+) or D (*chuA*+, *yjaA*-).[2] The primer sequences (Bioneer, Daejeon, South Korea) used in PCR, and expected size of products are presented in Table 1. *Escherichia coli* strain ECOR 62 was employed as positive control, and sterile distilled water as negative control.

Detection of β-lactamase genes. Genes encoding TEM, SHV and CTX-M-15 enzymes were detected by uniplex PCR assay on genomic DNA extracted as described above. The primer sequences used for amplification of the genes are shown in Table 1.[18,19] In PCR assay; the final 25 µL reaction mixture contained 2 mM of $MgCl_2$, 1X PCR buffer, 1 U of *Taq* DNA polymerase (Fermentas, Waltham, USA), 50 pmol of each primer, 200 mM of each dNTPs (Fermentas) and 2 µL of template DNA. Amplification program for bla_{TEM} and bla_{SHV} was performed as initial denaturation (94 ˚C, 3 min), 35 cycles each consisted of denaturation (94 ˚C, 30 sec), annealing (50 ˚C, 30 sec), extension (72 ˚C, 2 min) and final extension (72 ˚C, 10 min); but the program for $bla_{CTX\text{-}M}$ and $bla_{CTX\text{-}M\text{-}15}$ genes was initial denaturation (94 ˚C, 5 min), 30 cycles each was comprised of denaturation (94 ˚C, 30 sec), annealing (60 ˚C, 30 sec), extension (72 ˚C, 30 sec) and final extension (72 ˚C, 10 min). The amplification products were analyzed by electrophoresis in 1% agarose gel. Two standard strains were used as positive controls: *E. coli* ATCC 35218 for bla_{TEM}, and *Klebsiella pneumonia* ATCC 700603 for bla_{SHV} and $bla_{CTX\text{-}M\text{-}15}$. Strain of *E. coli* ATCC 25922 was employed as negative control. Also, a 100 bp DNA marker was used in all electrophoresis for determining the PCR product size.

Statistical analysis. Statistical analyses were performed using SPSS software (version 20.0; IBM, Armonk, USA). The Chi-square and Fisher's exact tests were used to evaluate the differences between fecal and colisepticemic isolates, and the significance of differences was set at $p < 0.05$.

Results

In this study, the 150 *E. coli* isolates belonged to four phylo-groups, including 78 isolates (52.00%) to A, 14 isolates (9.33%) to B1, 15 isolates (10.00%) to B2, and 43 isolates (28.67%) to phylo-group D (Table 2).

The phylo-genetic typing of 75 fecal isolates showed that they fell into four phylo-groups: 41 isolates (54.67%) into A, 9 isolates (12.00%) into B1, 5 isolates (6.67%) into B2, and 20 isolates (26.67%) into phylo-group D. Out of 75 septicemic isolates, 37 isolates (49.33%) were classified as phylotype A, 5 (6.67%) as B1, 10 (13.33%) as B2, and 23 (30.67%) as D. The statistical analysis showed that there was no significant difference in the prevalence of phylo-groups between fecal and septicemic isolates ($p > 0.05$).

Table 2. Distribution of *Escherichia coli* isolates from healthy (n = 75) and septicemic (n = 75) broiler chickens in phylo-genetic groups.

Groups	Healthy isolates (%)	Septicemic isolates (%)
chuA-, TspE4.C2-	41 (54.67)	37 (49.33)
chuA-, TspE4.C2+	9 (12.00)	5 (6.67)
chuA+, yjaA+	5 (6.67)	10 (13.33)
chuA+, yjaA-	20 (26.67)	23 (30.67)

No significant differences were detected among the groups ($p > 0.05$).

The phenotypic screening for ESBL production detected 24 (16.00%) isolates from a total of 150 isolates as ESBL-producer; but in PCR testing, 72 (48.00%) isolates (35 fecal and 37 septicemic) were identified to carry ESBL genes. The bla_{TEM}, bla_{SHV}, and $bla_{CTX\text{-}M\text{-}15}$ genes were identified in isolates recovered from both fecal and septicemic samples, but their prevalence was not influenced by the type of the sample ($p > 0.05$). A single resistance gene was detected in 57 (38.00%) isolates, but 15 (10.00%) isolates showed two or three resistance

Table 1. Primer sequences used to amplify phylogenetic groups and β–lactamase genes by the PCR technique.

Gene	Target	Primer	Product size (bp)	Reference
chuA	—	F: GACGAACCAACGGTCAGGAT R: TGCCGCCAGTACCAAAGACA	279	Clermont *et al.*[2]
yjaA	—	F: TGAAGTGTCAGGAGACGCTG R: ATGGAGAATGCGTTCCTCAAC	211	Clermont *et al.*[2]
TspE4.C2	—	F: GAGTAATGTCGGGGCATTCA R: CGCGCCAACAAAGTATTACG	152	Clermont *et al.*[2]
bla_{TEM}	β-lactam	F: ATAAAATTCTTGAAGACGAAA R: GACAGTTACCAATGCTTAATC	1080	Sharma *et al.*[18]
bla_{SHV}	β-lactam	F: CACTCAAGGATGTATTGTG R: TTAGCGTTGCCAGTGCTCG	928	Sharma *et al.*[18]
$bla_{CTX\text{-}M\text{-}15}$	β-lactam	F: CGCTTTGCGATGTGCAG R: ACCGCGATATCGTTGGT	550	Pitout *et al.*[19]

genes. Out of 150 tested isolates, 28 (18.67%) isolates were positive for *bla*TEM gene, 8 (5.33%) isolates for *bla*SHV, and 52 (34.67%) isolates for *bla*CTX-M-15 (Table 3).

Table 3. Detailed prevalence of extended-spectrum β-lactamase genes in *Escherichia coli* isolates from healthy (n = 75) and septicemic (n = 75) broiler chickens.

Gene	Healthy isolates (%)	Septicemic isolates (%)
***bla*TEM**	11 (14.67)	6 (8.00)
***bla*SHV**	0 (0.00)	1 (1.33)
***bla*CTX-M-15**	17 (22.67)	22 (29.33)
***bla*TEM + *bla*SHV**	1 (1.33)	1 (1.33)
***bla*TEM + *bla*CTX-M-15**	3 (4.00)	5 (6.67)
***bla*SHV + *bla*CTX-M-15**	2 (2.67)	2 (2.67)
***bla*TEM + *bla*SHV + *bla*CTX-M-15**	1 (1.33)	0 (0.00)
Total	35 (46.67)	37 (49.33)

No significant differences were detected among the groups ($p > 0.05$).

The ESBL-positive isolates belonged to four phylo-groups A, B1, B2, and D. Out of 72 positive isolates, 32 (44.44%) were allocated to phylo-group A, 10 (13.88%) to B1, 10 (13.88%) to B2, and 20 (27.78%) to D; but in detailed explanation, the *bla*TEM gene was not identified in phylo-group B2 of fecal isolates, and the *bla*SHV gene was not detected in phylo-groups B1 and B2 of septicemic isolates (Table 4).

Discussion

Escherichia coli strains are mainly assigned to four phylo-groups A, B1, B2 and D.[2] Phylotyping analyses have shown that commensal strains usually belong to groups A and B1, whereas the extraintestinal pathogenic strains belong mainly to groups B2 and D.[20,21] In the current study, we found the prevalence of groups A and D both in fecal and septicemic isolates, but their prevalence was not affected by the type of isolate ($p > 0.05$). The predominance of phylo-group A in fecal samples has been reported in previous works conducted in Iran and other countries as well.[22-25]

In contrast, Escobar-Páramo *et al.* analyzing fecal strains isolated from birds, non-human mammals and humans observed the prevalence of groups D and B1 in birds, A and B1 in non-human mammals, and A and B2 in humans.[26] The higher prevalence of phylo-groups A and D among septicemic isolates is in agreement with what have been reported previously; Rodriguez-Siek *et al*. divided APEC isolates from chickens into phylo-groups A (38.00%), B1 (15.00%), B2 (18.50%), and D (28.00%).[27] Dissanayake *et al.* indicated that APEC strains isolated from septicemic broilers and layers were mostly distributed in phylo-groups A (71.00%), followed by D (18.70%), B2 (7.90%), and B1 (4.10%).[25] Ghanbarpour *et al.* found that *E. coli* isolates recovered from colisepticemic broilers belonged to phylo-groups A (44.70%), B1 (21.30%), B2 (8.50%), and D (25.50%).[22] A study on distribution of APEC strains from Japanese quails in Southeast of Iran, Salehi and Ghanbarpour identified 55.00% of the isolates as A, 18.30% as B1, 17.40% as B2 and 9.20% as phylo-group D.[28] Hassani *et al.* analyzing the genomic background of *E. coli* recovered from broilers with *colibacilosis* in Northwest of Iran segregated the isolates in four phylo-groups: A (50.00%), B1 (2.80%), B2 (1.30%), and D (46.00%).[29] Therefore, the results obtained from this study and previous works imply that *E. coli* strains isolated from septicemic cases are likely the typical commensals which have been become virulent by acquisition of virulence genes from pathogenic types or through random functional point mutations.[30,31]

Recent studies have demonstrated a drastic increase in the prevalence of ESBLs among bacteria of the family *Enterobacteriaceae*. In the current study, 48.00% of the tested isolates contained one or more ESBL genes. This result is similar to what have been reported by others though there are some contrasts which could be due to differences in type of sample, isolation and testing methods, and geographical area. In Belgium, Smet *et al.* identified ESBL-producing *E. coli* isolates in 45.00% of cloacal samples from five broiler farms.[32] Out of 26 food samples of chicken or turkey origin analyzed in Tunisia, 7 (26.92%) samples carried *E. coli* with phenotype ESBL.[15] In comparison with our results, a higher prevalence of ESBL-producing *E. coli* isolates (22 out of 26 farms) was observed in cloacal swabs collected from Dutch broilers. In the same study, six of 18 broiler farmers carried isolates containing ESBL genes.[33] Also, in a study on samples

Table 4. Distribution of extended-spectrum β–lactamase genes in different phylo-groups of *Escherichia coli* isolates from broiler chickens.

Type of isolates	Gene	Number of positive isolates/total isolates in each phylo-group (%)			
		chuA-, TspE4.C2-	***chuA-, TspE4.C2+***	***chuA+, yjaA+***	***chuA+, yjaA-***
Fecal (n = 75)	*bla*TEM	7/41 (17.07)	2/9 (22.22)	0/5 (0.00)	7/20 (35.00)
	*bla*SHV	1/41 (2.44)	1/9 (11.11)	1/5 (20.00)	1/20 (5.00)
	*bla*CTX-M-15	10/41 (24.40)	5/9 (55.56)	2/5 (40.00)	6/20 (30.00)
Total		14/41 (34.15)	7/9 (77.78)	2/5 (40.00)	12/20 (60.00)
Septicemic (n = 75)	*bla*TEM	2/37 (5.41)	2/5 (40.00)	3/10(30.00)	5/23 (21.74)
	*bla*SHV	2/37 (5.41)	0/5 (0.00)	0/10 (0.00)	2/23 (8.70)
	*bla*CTX-M-15	16/37 (43.24)	3/5 (60.00)	6/10 (60.00)	4/23 (17.39)
Total		18/37 (48.65)	3/5 (60.00)	8/10 (80.00)	8/23 (34.78)

recovered from healthy broiler chicken in Germany, 88.60% of carcasses and 72.50% of ceca were positive for ESBL producers, which most of them were identified as *E. coli*.[34] Overdevest *et al.* found that 76.80% of chicken meat samples collected in the Netherlands contained ESBL-producing *E. coli* isolates. They also reported that 39 (69.60%) *E. coli* isolates recovered from rectal swabs of hospitalized patients were positive for ESBL.[35] Extended-spectrum β-lactamase-producing *Enterobacteriaceae*, in particular *E. coli*, are isolated with increasing frequency from human and other animal samples as well. In a study performed on isolates originated from humans (n = 183), dogs (n = 77), cats (n = 11) and horses (n = 100), Schmiedel *et al.* found that 83.60% of the human isolates and 91.60% of the animal isolates were ESBL-producers, and concluded that multi-resistant *Enterobacteriaceae* could be disseminated among human and animal populations.[36]

The epidemiology of ESBL genes is changing rapidly.[13] During the 1990s, most reports on ESBLs involved TEM/ SHV type enzymes, but the enzymes of CTX-M type have become the most prevalent family of ESBLs among *Enterobacteriaceae* since their first report in 1986.[37] In our study, $bla_{\text{CTX-M-15}}$ was the most common genotype (34.67%), followed by bla_{TEM} (18.67%) and bla_{SHV} (5.33%), which are in accordance with previous studies. Bagheri *et al.* isolated 204 *E. coli* strains from the external and internal cavity surfaces of broiler chicken carcasses in a slaughterhouse in Kerman province (Southeast Iran), and found that 27 (13.24%) and 4 (1.96%) isolates harbored the bla_{TEM} and bla_{SHV} genes, respectively.[38] Momtaz *et al.* reported a relatively high prevalence of non-β-lactam antibiotic resistance genes in 57 *E. coli* isolates recovered from chicken meat samples in an abattoir in Shahrekord in Iran, but could not find any positive isolates for bla_{SHV} gene.[39] A study on the presence of ESBL genes in 22 ampicilin-resistant *E. coli* isolates from feces of healthy broilers revealed that 17 (77.27%) isolates were positive for bla_{TEM} gene, but none of them showed a positive reaction for bla_{SHV} gene.[40] Out of 51 ESBL-producing *E. coli* isolates recovered from Belgian broilers and characterized by PCR, Smet *et al.* detected bla_{TEM} and $bla_{\text{CTX-M}}$ type genes in 26 (50.98%) and 22 (43.14%) isolates, respectively, but none of the isolates carried bla_{SHV} gene.[33] In Portugal, 38.2% of fecal samples obtained from broiler chickens in a slaughter-house were positive for ESBLs of the TEM and CTX-M groups.[41] In the United States and Germany, the predominant ESBL subtype in human isolates was reported to be CTX-M-15.[36,42]

Regarding to the distribution of ESBL genes in different phylo-groups, the resistance genes were segregated in phylo-groups A (32 isolates), B1 (10 isolates), B2 (10 isolates) and D (20 isolates), which is in accord with other reports. For example, Bagheri *et al.* reported that the 27 bla_{TEM} positive *E. coli* isolates recovered from chicken carcasses belonged to phylo-groups: A (14 isolates), B1 (six isolates), B2 (two isolates) and D (five isolates), but all of the four bla_{SHV} positive isolates belonged to phylo-group A.[38] In Portugal, a study on *E. coli* isolates recovered from raw chicken carcasses and feces of healthy chickens and swine indicated that the ESBL-positive isolates were distributed in a descending order in phylo-groups A, B1, and D.[43] In the study performed by Slama *et al.* in Tunisia, the 13 ESBL-positive *E. coli* isolates recovered from food samples belonged to phylo-groups A (nine isolates) or D (four isolates).[15]

In conclusion, our study showed a relatively high diversity of ESBL genes among commensal and pathogenic *E. coli* isolates from broiler chickens in Southwest of Iran, which could be of a great concern to animal and public health. Although the use of β-lactam antibiotics in chickens is unusual, the possibility of cross-selection with other antimicrobials used in poultry (such as sulfonamides and tetracyclines) may explain this result and should be further analyzed in future.

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References

1. Livermore DM. β-Lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995; 8: 557-584.
2. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 2000; 66(10): 4555-4558.
3. Gordon DM. The influence of ecological factors on the distribution and genetic structure of *Escherichia coli*. EcoSal Plus 2004; doi: 10.1128/ecosal plus.6.4.1.
4. Johnson JR, Delavari P, Kuskowski M, et al. Phylo-genetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. J Infect Dis 2001; 183(1): 78-88.
5. Gordon DM, Cowling A. The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: host and geographic effects. Microbiology 2003; 149(12): 3575-3586.
6. Maturana VG, de Pace F, Carlos C, et al. Subpathotypes of avian pathogenic *Escherichia coli* (APEC) exist as defined by their syndromes and virulence traits. Open Microbiol J 2011; 5(Suppl 1-M2): 55-64.
7. Kim TE, Jeong YW, Cho SH, et al. Chronological study of antibiotic resistances and their relevant genes in Korean avian pathogenic *Escherichia coli* isolates. Am Soc Microbiol 2007; 45(10): 3309-3315.

8. Altekruse SF, Elvinger, F, Lee, NK, et al. Antimicrobial susceptibilities of *Escherichia coli* strains from a turkey operation. J Am Vet Med Assoc 2002; 221(3): 411-416.
9. Watts JL, Salmon SA, Yancey RJ, et al. Minimum inhibitory concentration of bacteria isolates from septicemia and airsacculitis in ducks. J Vet Diagn Invest 1993; 5(4): 625-628.
10. Bradford PA. Extended-spectrum β-lactamases in the 21st century: Characterization epidemiology and detection of this important resistance threat. Clin Microbiol Rev 2001; 14(4): 933-951.
11. Ambler RP. The structure of β-lactameses. Philos Trans R Soc London B [Biol] 1980; 289 (1036): 321-331.
12. Jacoby G, Munoz-Price LS. The new β-lactamases. New Engl J Med 2005; 352(4): 380-391.
13. Hawkey PM, Jones AM. The changing epidemiology of resistance. J Antimicrob Chemother 2009; 64 (Suppl 1): 3-10.
14. Schwaber MJ, Navon-Venezia S, Schwartz D, et al. High levels of antimicrobial coresistance among extended-spectrum β-lactamase-producing *Enterobacteriaceae.* Antimicrob Agents Chemother 2005; 49(5): 2137-2139.
15. Ben Slama K, Jouini A, Ben Sallem R, et al. Prevalence of broad-spectrum cephalosporin-resistant *Escherichia coli* isolates in food samples in Tunisia, and characterization of integrons and antimicrobial resistance mechanisms implicated. Int J Food Microbiol 2010; 137(2-3): 281-286.
16. Quinn PJ, Carter ME, Markey B, et al. Clinical veterinary microbiology. St. Louis, USA: Mosby 1994; 209-226.
17. CLSI. Performance standards for antimicrobial susceptibility testing. 22nd Informational Supplement. M100-S22, 32(3). Wayne, Pennsylvania: Clinical and Laboratory Standards Institute, 2012; 50-51.
18. Sharma J, Sharma M, Ray P. Detection of TEM and SHV genes in *Escherichia coli* and *Klebsiella pneumonia* isolates in a tertiary care hospital from India. Indian J Med Res 2010; 132: 332-336.
19. Pitout JD, Hossain A, Hanson ND. Phenotypic and molecular detection of CTX-M β-lactamases produced by *Escherichia coli* and *Klebsiella spp*. J Clin Microbiol 2004; 42(12): 5715-5721.
20. Bingen E, Picard B, Brahimi N, et al. Phylogenetic analysis of *Escherichia coli* strains causing neonatal meningitis suggests horizontal gene transfer from a predominant pool of highly virulent B2 group strains. J Infect Dis 1998; 177(3): 642-650.
21. Picard B, Garcia JS, Gouriou S, et al. The Link between phylogeny and virulence in *Escherichia coli* extra-intestinal infection. Infect Immun 1999; 67(2): 546-553.
22. Ghanbarpour R, Sami M, Salehi, M, et al. Phylogenetic background and virulence genes of *Escherichia coli* isolates from colisepticimic and healthy broiler chickens in Iran. Trop Anim Health Prod 2011; 43(1): 153-157.
23. Higgins J, Hohn C, Hornor S, et al. Genotyping of *Escherichia coli* from environmental and animal samples. J Microbiol Meth 2007; 70(2): 227-235.
24. Ewers C, Li G, Wilking H, et al. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: how closely related are they? Int J Med Microbiol 2007; 297(3): 163-176.
25. Dissanayake DRA, Wijewardana TG, Gunawardena GA, et al. Distribution of lipopolysaccharide core types among avian pathogenic *Escherichia coli* in relation to the major phylogenetic groups. Vet Microbiol 2008; 132(3-4):355-363.
26. Escobar-Páramo P, LeMenach A, LeGall T, et al. Identification of forces shaping the commensal *Escherichia coli* genetic structure by comparing animal and human isolates. Environ Microbiol 2006; 8(11): 1975-1984.
27. Rodriguez-Siek KE, Giddings CW, Doetkott C, et al. Characterizing the APEC pathotype. Vet Res 2005; 36(2): 241-256.
28. Salehi M, Ghanbarpour R. Phenotypic and genotypic properties of *Escherichia coli* isolated from colisepticemic cases of Japanese quail. Trop Anim Health Prod 2010; 42(7): 1497-1504.
29. Hassani B, Shayegh J, Ameghi A, et al. Phylogenetic typing of *Escherichia coli* isolated from broilers with colibacillosis in Tabriz, Northwest of Iran. Arch Razi Inst 2013; 68(1): 43-46.
30. Ochman H, Lawrence JG, Groisman EA. Lateral gene transfer and the nature of bacterial innovation. Nature 2000; 405: 299-304.
31. Sokurenko EV, Chesnokova V, Dykhuizen DE, et al. Pathogenic adaptation of *Escherichia coli* by natural variation of the FimH adhesin. Proc Natl Acad Sci 1998; 95(15): 8922-8926.
32. Smet A, Martel A, Persoons D, et al. Diversity of Extended-spectrum β-lactamases and class C β-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. Antimicrob Agents Chemother 2008; 52(4): 1238-1243.
33. Dierikx C, Van der Goot J, Fabri T, et al. Extended-spectrum β-lactamase and Ampc β-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J Antimicrob Chemother 2013; 68(1): 60-67.
34. Reich F, Atanassova, V, Klein, G. Extended-spectrum β-lactamase and Ampc-producing Enterobacteria in healthy broiler chickens, Germany. Emerg Infect Diseases 2013; 19(8): 1253-1259.
35. Overdevest I, Willemsen I, Rijinsburger M, et al. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg Infect Diseases 2011; 17(7): 1216-1222.
36. Schmiedel J, Falgenhauer, L, Domann E, et al. Multi-resistant extended-spectrum β-lactamase-producing

Enterobacteriaceae from humans companion animals and horses in central Hesse, Germany. BMC Microbiology 2014; 14: 187-199.
37. Smet A, Van Nieuwerburgh F, Vandekerckhove TTM, et al. Complete nucleotide sequence of CTX-M-15 plasmids from clinical *Escherichia coli* isolates: Insertional events of transposons and insertion sequences. PLoS One 2010; 5(6): 1-8.
38. Bagheri M, Ghanbarpour R, Alizade H. Shiga toxin and beta-lactamases genes in *Escherichia coli* phylotypes isolated from carcasses of broiler chickens slaughtered in Iran. Int J Food Microbiol 2014; 177: 16-20.
39. Momtaz H, Rahimi E, Moshkelani S. Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. Vet Med 2012; 57(4): 193-197.
40. Brinas L, Zarazaga M, Saenz Y, et al. β-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob Agents Chemother 2002; 46(10): 3156-3163.
41. Costa D, Vinue L, Poeta P, et al. Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* isolates in fecal samples of broilers. Vet Microbiol 2009; 138(3-4): 339-344.
42. Xu L, Shabir S, Bodah T, et al. Regional survey of CTX-M type extended-spectrum β-lactamses among *Entero-bacteriaceae* reveals heterogenecity in the distribution of the ST131 clone. J Antimicrob Chemother 2011; 66(3): 505-511.
43. Machado E, Coque TM, Canton R, et al. Antibiotic resistance integrons and extended-spectrum β-lactamases among *Enterobacteriaceae* isolates recovered from chickens and swine in Portugal. J Antimicrob Chemother 2008; 62(2): 296-302.