Shiga toxin-producing *Escherichia coli* isolated from chicken meat in Iran: Serogroups, virulence factors, and antimicrobial resistance properties

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ABSTRACT The aim of the current study was to determine the virulence factors, serogroups, and antibiotic resistance properties of Shiga toxin-producing *Escherichia coli* isolated from chicken meat samples. A total of 422 chicken meat samples were collected from 5 townships of Iran. Specimens were immediately transferred to the laboratory in a cooler with an ice pack. Samples were cultured, and the positive culture samples were analyzed by PCR assays. Finally, the antimicrobial susceptibility test was performed using the disk diffusion method in Mueller–Hinton agar. According to the results, out of 422 samples, 146 (34.59%) were confirmed to be *E. coli* positive and among *E. coli*-positive samples, 51 (34.93%) and 31 (21.23%) were from attaching and effacing *E. coli* (AEEC) and enterohemorrhagic *E. coli* (EHEC) subgroups, respectively. All of the EHEC-positive samples had all *stx1*, *eaeA*, and *ehly* virulence genes, whereas only 5 (9.80%) of AEEC subgroup had all *stx1*, *stx2*, and *eaeA* genes. As the data revealed, O157 was the most prevalent and O111 was the least prevalent strains in the Shiga toxin-producing *E. coli* (STEC) population. Among STEC strains, *sulI* and *blaSHV* had the highest and lowest incidence rate, respectively. There was a high resistance to tetracycline (76.82%), followed by chloramphenicol (73.17%) and nitrofurantoin (63.41%), but there was low resistance to cephalotine (7.31%) antibiotics in isolated strains. Results shows that the PCR technique has a high performance for detection of serogroups, virulence genes, and antibiotic resistance genes in STEC strains. This study is the first prevalence report of detection of virulence genes, serogroups, and antibiotic resistance properties of STEC strains isolated from chicken meat samples in Iran. Based on the results, chicken meat is one of the main sources of STEC strains and its virulence factors in Iran, so an accurate meat inspection would reduce disease outbreaks.

Key words: Shiga toxin-producing *Escherichia coli*, serogroup, molecular characterization, chicken meat, Iran

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INTRODUCTION

Escherichia coli is a gram-negative, rod-shaped, flagellated, nonsporulating, and facultative anaerobic bacterium that belongs to *Enterobacteriaceae* family. Some serogroups of *E. coli* are able to cause disease and food poisoning. These types of *E. coli* are generally classified into 6 subgroups including enterotoxigenic *E. coli* (**ETEC**), enteropathogenic *E. coli* (**EPEC**), enteroinvasive *E. coli*, enterohemorrhagic *E. coli* (**EHEC**), enteroadherent *E. coli*, and diffusely adherent *E. coli* (Holko et al., 2006). The EHEC strains are one of the subsets of Shiga toxin (**Stx**)-producing *E. coli* (**STEC**) strains, which are isolated from patients and are responsible for severe clinical symptoms such as hemorrhagic colitis (**HC**) and the potentially lethal hemolytic uremic syndrome (**HUS**; Karch et al., 2005; Karmali et al., 2010). The STEC serogroups associated with human diseases are numerous and include O1, O2, O4, O5,O6, O22, O23, O26, O38, O45, O48, O50, O55, O73, O75, O91, O100, O103, O104, O105, O111, O113, O114, O115, O117, O118, O119, O121, O125, O126, O128, O132, O145, O153, O163, O165, and O166, as well as untypeable isolates (DebRoy et al., 2004; Heijnen and Medema, 2006; Erickson and Doyle, 2007; Lin et al., 2011). Several studies showed that consumption of contaminated food with STEC strains is the main cause of human infections (Mead et al., 1999; Caprioli et al., 2005; Hussein and Sakuma, 2005). It seems that STEC virulence genes have a major role in causing diseases. Shiga toxins, the main virulence factors contributing to pathogenicity, consist of 2 major types, the *Stx1*, which is identical to *Stx* of *Shigella dysenteriae*, and *Stx2*, which is 56% homologous to *Stx1* (Scheutz and Strockbine, 2005). On the other hand, the *Stx* is a family of cytotoxic proteins that consists of an ap-

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proximately 32-kDa A subunit, with *N*-glycosidase activity, noncovalently associated with a pentamer of B subunits (7.7 kDa each), which mediate binding to specific receptor molecules (O'Loughlin and Robins-Browne, 2001). The *Stx* genes are carried by temperate lambdoid bacteriophages, along with late genes of the phage genome, and are regulated by the phage regulatory circuits (Schmidt, 2001; Waldor and Friedman, 2005). Induction of phage by agents that affect bacterial DNA or the cell wall, including antibiotics, can lead to a massive increase in toxin production (Kimmitt et al., 2000). The *Stx1*, but not *Stx2* and its variants, is iron regulated, with enhanced synthesis occurring in low-iron environments.

Additional factors that contribute to virulence have also been described, including intimin (encoded by the *eaeA* gene), an outer membrane protein involved in the attachment of *E. coli* to the enterocyte, and EHEC hemolysin (encoded by EHEC *hlyA*), which acts as a pore-forming cytolysin and causes damage to cells (Paton and Paton, 1998). On the other hand, a critical component is the gene that encodes the outer membrane protein intimin, which functions as an adhesin. Variation in the amino acid sequence in the C-terminus of intimin has led to recognition of at least 21 variants of intimin in STEC and EPEC. A factor that may also affect the virulence of STEC strains is the enterohemolysin (*Ehly*), also called enterohemorrhagic *E. coli* hemolysin (EHEC-*HlyA*), which is encoded by the *ehxA* gene (Schmidt et al., 1995). The *Ehly* operon is positively regulated by *GrlA*, a LEE-encoded positive regulator of the LEE operon (Saitoh et al., 2008). Antibody to *Ehly* is produced in humans with EHEC disease and experimental data suggest that the hemolysin may aid survival of STEC in the intestine by making iron available from lysis of erythrocytes.

The genes located in the genome of temperate bacteriophages encode 2 distinct *stx* genes. The Stx1 and Stx2 toxins possess similar biological activities, including cytotoxicity to Vero and HeLa cells, but are different immunologically (Law, 2000). Both toxins are composed of an enzymatically active A subunit and a pentameric B subunit (Paton et al., 1999; Law, 2000). The intimin encoded by the *eae* gene located in the chromosomal locus of enterocyte effacement is involved in the intimate attachment of bacteria to enterocytes (Schmitt et al., 1991). However, some STEC involved in severe diseases do not contain the genetic information encoding intimin. Plasmid-encoded virulence factors are also probably involved in the pathogenicity of STEC (Law, 2000).

Basically, hemolysins are encoded by polycistronic operons, which consist of 4 genes arranged in the order of *hly-CABD* (Schmidt et al., 1996; Stanley et al., 1998). The product of the *hlyC* gene is involved in activation of the hemolytic toxin, the product of the *hlyA* gene. The gene products of *hlyB* and *hlyD* together with *TolC* are involved in secretion of the hemolysin through the bacterial cell wall (Holland et al., 2005). α-Hemolysin is a pore-forming cytolysin and serves as a virulence factor in intestinal and extraintestinal pathogenic strains of *E. coli*. It was suggested that the genes encoding α-hemolysin (*hlyCABD*), which can be found on the chromosome and plasmid, were acquired through horizontal gene transfer. Plasmid-encoded α-*hly* is associated with certain ETEC, STEC, and EPEC strains (Burgos and Beutin, 2010).

The diseases that cause by *E. coli* need persistent antibiotic therapy, but many studies have been reported the occurrence of antibiotic resistance (van den Bogaard and Stobberingh, 1999; Galland et al., 2001). Antibiotic resistance can cause more severe diseases in humans and animals. Antibiotic resistance in STEC strains is associated with the presences of some antibiotic resistance genes (Galland et al., 2001). Chicken meat is one of the most popular foods among the Iranian population. However; the epidemiology and prevalence of STEC strains in chicken meat is essentially unknown. Therefore, this study was carried out for molecular characterization and evaluation of antibiotic resistance properties in STEC serogroups isolated from chicken meat in Iran.

MATERIALS AND METHODS

Samples

A total of 422 chicken meat samples were randomly purchased from the supermarkets in various parts of Iran including Shahrekord (n = 76), Isfahan (n = 102), Kermanshah (n = 81), Yasuj (n = 80), and Ahvaz (n = 83) townships from March 2010 to March 2011. All samples were from 4-wk-old broiler chickens reared in an aviculture. In the present study, the chicken breast muscle was used. The external surfaces of chicken meats were disinfected with 70% alcohol to minimize surface contamination. The pieces of the muscles were collected separately into sterile bags using sterile scissors and tissue forceps. All samples were immediately transported to the laboratory in cooled boxes. All techniques were applied to prevent cross-contamination within and between townships.

The surfaces of chicken muscles were seared with a hot sterile spatula. Then the muscles were incised and cultured on 5% sheep blood and MacConkey agar (Merck, Germany) and incubated for 18 to 24 h at 37°C. Colonies with the typical color and appearance of *E. coli* were picked and streaked again on blood agar plates and restreaked on EMB agar (Merck, Darmstadt, Germany). The green metallic sheen colonies were considered to be *E. coli*. The presumptive colonies were biochemically tested for growth on triple sugar iron agar and lysine iron agar, oxidative/fermentative degradation of glucose, citrate utilization, urease production, indol fermentation, tryptophan degradation,

glucose degradation (methyl red test), and motility. The *E. coli* isolates were kept in LB/glycerol at −70°C (Mooljuntee et al., 2010). The colonies were confirmed using PCR based on the detection of *16S* rRNA gene region of *E. coli* described by Sabat et al. (2000).

DNA Isolation

Bacterial strains were subcultured overnight in Luria-Bertani broth (Merck), and genomic DNA was extracted using a DNA extraction kit (DNP, CinnaGen, Tehran, Iran) according to the manufacturer's instructions.

Detection of Serogroups, Virulence Factors, and Antibiotic Resistance Genes of Stx-Producing E. coli

The results of culture method were studied using several PCR methods. Table 1 shows the primers used for detection of Stx-producing *E. coli* serogroups, virulence genes, and antimicrobial resistant genes. A DNA thermo-cycler (Eppendorf Mastercycler, Eppendorf-Nethel-Hinz GmbH, Hamburg, Germany) was used in all PCR reactions. The amplified products were visualized using ethidium bromide staining after gel electrophoresis of 10 μL of the final reaction mixture in 1.5% agarose.

Table 1. Primers used for detection of Shiga toxin-producing *Escherichia coli* serogroups, virulence genes, and antimicrobial resistant genes

Primer name	Sequence	Size of product (bp)	Target gene	Reference
O26F	CAGAATGGTTATGCTACTGT	423	*wzx*	Possé et al., 2007
O26R	CTTACATTTGTTTTCGGCATC			
O103F	TTGGAGCGTTAACTGGACCT	321	*wzx*	Possé et al., 2007
O103R	GCTCCCGAGCACGTATAAG			
O111F	TAGAGAAATTATCAAGTTAGTTCC	406	*wzx*	Possé et al., 2007
O111R	ATAGTTATGAACATCTTGTTTAGC			
O145F	CCATCAACAGATTTAGGAGTG	609	*wzx*	Possé et al., 2007
O145R	TTTCTACCGCGAATCTATC			
O157F	CGGACATCCATGTGATATGG	259	*wzx*	Possé et al., 2007
O157R	TTGCCTATGTACAGCTAATCC			
O45F	CCGGGTTTCGATTTGTGAAGGTTG	527	*wzx1*	DebRoy et al., 2005
O45R	CACAACAGCCACTACTAGGCAGAA			
O91F	GCTGACCTTCATGATCTGTTGA	291	*gnd*	Perelle et al., 2002
O91R	TAATTTAACCCGTAGAATCGCTGC			
O113F	GGGTTAGATGGAGCGCTATTGAGA	771	*wzx*	DebRoy et al., 2004
O113R	AGGTCACCCTCTGAATTATGGCAG			
O121F	TGGCTAGTGGCATTCTGATG	322	*wzx*	Fratamico et al., 2003
O121R	TGATACTTTAGCCGCCCTTG			
O128F	GCTTTCTGCCGATATTTGGC	289	*galF*	Shao et al., 2003
O128R	CCGACGGACTGATGCCGGTGATT			
Stx1F	AAATCGCCATTCGTTGACTACTTCT	366	*stx1*	Brian et al., 1992
Stx1R	TGCCATTCTGGCAACTCGCGATGCA			
Stx2F	CGATCGTCACTCACTGGTTTCATCA	282	*stx2*	Brian et al., 1992
Stx2R	GGATATTCTCCCCACTCTGACACC			
EAE1	TGCGGCACAACAGGCGGCGA	629	*eaeA*	Heuvelink et al., 1995
EAE2	CGGTCGCCGCACCAGGATTC			
Hly F	CAATGCAGATGCAGATACCG	432	*ehly*	Idress et al., 2010
Hly R	CAGAGATGTCGTTGCAGCAG			
Streptomycin1	TATCCAGCTAAGCGCGAACT	447	*aadA1*	Randall et al., 2004
Streptomycin2	ATTTGCCGACTACCTTGGTC			
Tetracycline1	GGTTCACTCGAACGACGTCA	577	*tetA*	Randall et al., 2004
Tetracycline2	CTGTCCGACAAGTTGCATGA			
Tetracycline3	CCTCAGCTTCTCAACGCGTG	634	*tetB*	Randall et al., 2004
Tetracycline4	GCACCTTGCTGATGACTCTT			
Trimethoprim1	GGAGTGCCAAAGGTGAACAGC	367	*dfrA1*	Toro et al., 2005
Trimethoprim2	GAGGCGAAGTCTTGGGTAAAAAC			
Fluoroquinolone1	GGGTATGGATATTATTGATAAAG	670	*qnr*	Mammeri et al., 2005
Fluoroquinolone2	CTAATCCGGCAGCACTATTTA			
Gentamicin1	CTTCAGGATGGCAAGTTGGT	286	*aac(3)-IV*	Van et al., 2008
Gentamicin2	TCATCTCGTTCTCCGCTCAT			
Sulfonamide1	TTCGGCATTCTGAATCTCAC	822	*sul1*	Van et al., 2008
Sulfonamide2	ATGATCTAACCCTCGGTCTC			
Cephalothin1	TCGCCTGTGTATTATCTCCC	768	*blaSHV*	Van et al., 2008
Cephalothin2	CGCAGATAAATCACCACAATG			
Ampicillin1	TGGCCAGAACTGACAGGCAAA	462	*CITM*	Van et al., 2008
Ampicillin2	TTTCTCCTGAACGTGGCTGGC			
Chloramphenicol1	AGTTGCTCAATGTACCTATAACC	547	*cat1*	Van et al., 2008
Chloramphenicol2	TTGTAATTCATTAAGCATTCTGCC			
Chloramphenicol3	CCGCCACGGTGTTGTTGTTATC	698	*cmlA*	Van et al., 2008
Chloramphenicol4	CACCTTGCCTGCCCATCATTAG			

Table 2. Prevalence of *Escherichia coli* isolated from chicken meat in different townships

Township	No. of samples	No. of positive samples (%)
Shahrekord	76	16 (3.79)[a]
Isfahan	102	33 (7.81)
Kermanshah	81	32 (7.58)
Yasouj	80	29 (6.87)
Ahvaz	83	36 (8.53)[b]
Total	422	146 (34.59)

[a,b]Dissimilar superscripts within a column indicate significant differences, $P < 0.05$.

Strains of *E. coli* O157:K88ac:H19, CAPM 5933, and *E. coli* O159:H20, CAPM 6006 were used as positive controls.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed by the Kirby–Bauer disc diffusion method using Mueller–Hinton agar (HiMedia Laboratories, Mumbai, India), according to the Clinical and Laboratory Standards Institute guidelines. After incubating the inoculated plate aerobically at 37°C for 18 to 24 h in an aerobic atmosphere, the susceptibility of the *E. coli* isolates to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by Clinical and Laboratory Standards Institute (2006). *Escherichia coli* ATCC 25922 was used as quality control organism in antimicrobial susceptibility determination.

Statistical Analysis

Statistical analysis was performed using SPSS/16.0 software (SPSS Inc., Chicago, IL) for significant relationships. The incidences of serogroups, virulence factors, and antibiotic resistance properties of *E. coli* isolated from chicken meat samples were statistically analyzed. Statistical significance was regarded at a P value <0.05.

RESULTS

The study population consisted of 422 chicken meats. As shown in Table 2, 146 (34.59%) samples were confirmed to be *E. coli* positive. There were significant differences ($P < 0.05$) between presence of *E. coli* in Shahrekord and Ahvaz city. The highest prevalence of *E. coli* in chicken meat occurred in Ahvaz and the lowest in Shahrekord. Table 3 indicates the distribution of virulence factors of *E. coli* strains isolated from chicken meat in Iran. There were significant differences between the presence of attaching and effacing *E. coli* (**AEEC**) and EHEC subgroups in chicken meat samples ($P < 0.05$). All of the EHEC-positive samples had *stx1, eaeA*, and *ehly* virulence genes, whereas the presence of these genes in AEEC-positive samples was lower. Table 4 shows the total prevalence of STEC serogroups isolated from chicken meat in different townships. Based on the results, O157 had the highest incidence of serogroups, whereas O103 had the lowest incidence in STEC strains isolated from chicken meat samples in Iran. There were significant differences ($P < 0.01$) between the presence of O157 and O111, nondetected serogroups with O111 and O128 and nondetected serogroups with O45. Table 5 shows the distribution of virulence genes in STEC serogroups isolated from chicken meat samples. There were significant differences ($P < 0.01$) between the presence of *stx1* and *stx2* and between the presence of *stx1* with *ehly* and *eaeA* with *ehly* virulence genes. There were also significant differences ($P < 0.05$) between the presence of *stx1* and *eaeA*.

Table 6 revealed the antibiotic resistance properties distribution of STEC serogroups isolated from chicken meat in Iran. In total *sul1* had the highest (64.63%), whereas *blaSHV* had the lowest (3.65%) incidence of antibiotic resistance genes. Statistical analysis showed that resistance of O157 serogroup to sulfonamides had significant differences ($P < 0.05$) with β-lactams and ampicillin antibiotics. Resistance of O26 serogroup to enrofloxacin exhibited significant differences ($P < 0.05$) in β-lactams. Resistance of 103 serogroup to tetracycline, gentamicin, and ciprofloxacin had significant differences ($P < 0.05$) with cephalothin, ampicillin,

Table 3. Distribution of virulence factors in Shiga toxin-producing *Escherichia coli* serogroups isolated from chicken meat[1]

Subgroup	No. of positive samples (%)	Virulence gene, no. (%)
Nondetected	64 (43.83)	—
EHEC	31 (21.23)[a]	*stx1, eaeA, ehly*: 31 (100)
AEEC	51 (34.93)[b]	*stx1*: 49 (96.07)
		stx2: 5 (9.80)
		eaeA: 46 (90.19)
		stx1, eaeA: 38 (74.50)
		stx2, eaeA: 8 (15.68)
		stx1, stx2, eaeA: 5 (9.80)
Total	146 (34.59)	

[a,b]Dissimilar superscripts within a column indicate significant differences, $P < 0.05$.

[1]EHEC = enterohemorrhagic *E. coli*; AEEC = attaching and effacing *E. coli*.

β-lactams, and chloramphenicol. Resistance of O145 serogroup to chloramphenicol had significant differences ($P < 0.05$) with β-lactams, gentamicin, and cephalothin. Resistance of O91 serogroup to quinolones had significant differences ($P < 0.05$) with tetracycline and β-lactams. Resistance of O113 serogroup to sulfonamides and terimethoprim had significant differences ($P < 0.05$) with β-lactams, quinolones, cephalothin, lincomycin, ampicillin, and nitrofurantoin. Resistance of O121 serogroup to nitrofurantoin and chloramphenicol had significant differences ($P < 0.05$) with betalactams, ciprofloxacin, and lincomycin.

DISCUSSION

Escherichia coli isolates frequently contaminate food of animal origin; in our investigation, this microorganism was recovered from 146 out of 422 (34.59%) tested chicken meat samples, and most of isolates showed the multiple-resistant phenotypes. This survey revealed that Ahvaz city had the highest prevalence of STEC strains isolated from chicken meat samples. In addition, the *sul1* gene had the highest prevalence of antibiotic resistance genes. Our study showed that the STEC strains isolated from chicken meat had the highest antibiotic resistance to tetracycline. The results of the present study revealed that chicken meat can be easily contaminated by *E. coli*. Preparation of healthy chicken meat is very important. We recommend the PCR technique as an accurate, rapid, and safe method for inspection of chicken meat. Providing healthy food has been considered in many countries, including Taiwan (Chiueh et al., 2001), United States (Barkocy-Gallagher et al., 2003), Italy (Caprioli et al., 1993), Switzerland (al-Saigh et al., 2004), and Germany (Beutin et al., 1994). The high importance of *E. coli* as a cause of human poisoning has been shown in the mentioned countries.

Previous research showed that the STEC strains are mostly commensal bacteria in animals, with a high potential for foodborne transmission to humans (Caprioli et al., 2005). *Escherichia coli* has been isolated from wide ranges of raw foodstuffs including dairy cattle and their products (Hussein and Sakuma, 2005), beef cattle (Galland et al., 2001), milk (Bürk et al., 2002), meat products (Chapman et al., 2001), retail fresh meats and poultry (Doyle and Schoeni, 1987), and eggs (Chiueh et al., 2001).

Our results showed that O157 is the major STEC serogroup in poultry meat, which is similar to a previous study in Korea performed on raw ground poultry (Henry et al., 2001). The other study in Korea showed that 41 of 900 poultry samples (4.6%) were *E. coli* positive and there was no O157 serogroup detected (Lee et al., 2009). In another Czech study, out of 987 samples, 22 strains (2.2%) were identified as *E. coli* O157 and only 9 poultry meat samples were positive for all *stx1*, *stx2, eaeA*, and *ehxA* virlence genes (Lukásová et al., 2004). Our results showed that 100% of EHEC sero-

Table 4. Prevalence of Shiga toxin-producing *Escherichia coli* serogroups isolated from chicken meat in different townships

Township[1]	O157	O26	O103	O111	O145	O45	O91	O113	O121	O128	Nondetected
Shahrekord (16)	4	—	—	1	2	—	—	1	1	—	7
Isfahan (33)	9	1	1	—	1	—	5	1	2	1	12
Kermanshah (32)	8	2	1	—	1	1	2	1	2	—	14
Yasouj (29)	1	4	3	—	2	1	2	2	1	—	13
Ahvaz (36)	9	2	—	—	—	2	2	1	1	1	18
Total (146)[2]	31 (37.80)[a]	9 (10.97)	5 (6.09)	1 (1.21)[ab]	6 (7.31)	4 (4.87)[d]	11 (13.41)	6 (7.31)	7 (8.53)	2 (2.43)[c]	64 (43.83)[bcd]

[a–d]Dissimilar superscripts within a row indicate significant differences, $P < 0.01$.

[1]Numbers in parentheses in this column are numbers of positive samples in each province.

[2]Numbers in parentheses in this row are percentages.

Table 5. Distribution of virulence genes in Shiga toxin-producing *Escherichia coli* serogroups isolated from chicken meat

Serogroup[1]	*stx1*	*stx2*	*eaeA*	*ehly*
O157 (31)	31	—	31	31
O26 (9)	9	2	8	—
O103 (5)	5	—	4	—
O111 (1)	1	—	—	—
O145 (6)	6	1	5	—
O45 (4)	4	—	4	—
O91 (11)	11	2	10	—
O113 (6)	6	—	6	—
O121 (7)	6	—	7	—
O128 (2)	2	—	2	—
Total (82)[2]	80 (97.56)^{AB,b}	5 (6.09)^{A,ab}	77 (93.90)^{C,a}	31 (37.80)^{BC}

$^{A–C}$Dissimilar uppercase superscripts within a row indicate significant differences, $P < 0.01$.

^{a,b}Dissimilar lowercase superscripts within a row indicate significant differences, $P < 0.05$.

[1]Numbers in parentheses in this column are numbers of positive samples in each serogroup.

[2]Numbers in parentheses in this row are percentages.

groups were positive for all *stx1*, *eaeA*, and *ehly* genes; in addition, 9.8% of AEEC serogroups were positive for all *stx1*, *stx2*, and *eaeA* genes. In another investigation, *E. coli* was isolated from 48% (48/100) of chickens and all of these isolates had an *eaeA* virulence factor (Kilic et al., 2007) that was lower than our study (90.19%).

It is well documented that STEC strains vary in their capacity to cause serious diseases in humans or animals, and this is associated with the type or amount of *stx* produced (Law, 2000). Therefore, the type of *stx* toxin produced by STEC isolated from human infections has been extensively studied (Schmidt et al., 1995). In contrast, little is known about *stx2* subtype frequency and the combination of virulence factors expressed by STEC from the intestinal tract of healthy animals. Besides, the *ehly* virulence gene acts as a pore-forming cytolysin on eukaryotic cells. In addition to *stx* and *ehly* vrulence genes, the *eae* gene has been isolated from the cases of HUS and HC (Law, 2000). Our results confirmed the presence of virulence genes including *stx*, *eae*, and *ehly* in chicken meat. Therefore, the raw or undercooked consumption of these chicken meats can cause diseases such as HUS and HC in humans.

Because of the high indiscriminate use of antibiotics, especially in veterinary medicine, antibiotic resistance against most effective antibiotics has occurred. Several programs such as the DANMAP in Denmark and the JVARM5 in Japan (Heuer and Hammerun, 2005; Asai et al., 2006) have described the resistance to various antibiotic drugs in veterinary medicine. In this study, the levels of resistance to some antimicrobial agents (tetracycline, chloramphenicol, and nitrofurantoin) were high (63 to 77%). Therefore, the STEC strains that were isolated from poultry meat are a potential reservoir of antimicrobial resistance genes.

Chloramphenicol and nitrofurantoin are 2 forbidden antibiotics. The high presence of resistance to these 2 antibiotics indicated the irregular and unauthorized use of these drugs in veterinary treatment in Iran. Veterinarians in many veterinary fields such as large animal internal medicine, poultry, and even aquaculture use these antibiotics as a basic treatment. Therefore, in a very short period of time, antibiotic resistance will appear. In the same study in Thailand, *E. coli* isolates have 100% resistance to tetracycline, ampicillin, and erythromycin, whereas resistance to cephalothin and sulfonamide+trimethoprim was 73.3 and 26.7%, respectively (Mooljuntee et al., 2010). Another study showed 82.4% resistance of *E. coli* isolated from broiler chickens to tetracycline (Miles et al., 2006). Prescription of tetracycline is not recommended in cases of *E. coli* infection. To our knowledge, ciprofloxacin and enrofloxacin are common antibiotics used for *E. coli* infections, but our results showed 36.58 and 46.34% resistance to these drugs, respectively, which is in accordance with the previous study (82% resistance to ciprofloxacin; Akond et al., 2009). Another study showed that in some countries approximately 300,000 kg of antibiotics are used annually on veterinary prescriptions in animals, of which 10% is used in poultry (van den Bogaard, 2000). It seems that the high and irregular prescription of antibiotics is the major reason for the emergence of antibiotic resistance.

Our results showed *sul1* (64.63%) had a highest incidence of antimicrobial resistance genes, followed by *aadA1* (62.19%), *dfrA1* (58.53%), and *qnr* (56.09%), but research in Thailand showed that the presences of *tetA*, *CITM*, *ereA*, *SHV*, *sul1*, and *dhfrV* were 90, 93.3, 73.3, 86.4, 100, and 100%, respectively (Mooljuntee et al., 2010). A study in Kenya indicated that tetracycline (75.9%) and cotrimoxazole (72.4%) had the highest antibiotic resistances in *E. coli* isolated from slaughtered broiler chickens (Adelaide et al., 2008).

These differences in the levels of antibiotic resistance in various countries showed that the resistance properties of STEC strains closely depend on the geographical regions. On the other hand, the antibiotic resistances in STEC serogroups isolated from chicken meat samples closely depends on antibiotic prescriptions (FDA, 2009; APUA, 2010; Wayne et al., 2011). Prescription of antibiotics in various countries is related to common and conventional antibiotics that are available.

Table 6. Antimicrobial resistance properties in Shiga toxin-producing *Escherichia coli* serotypes isolated from chicken meat[1]

Antimicrobial resistance[2]	O157 (31)	O26 (9)	O103 (5)	O111 (1)	O145 (6)	O45 (4)	O91 (11)	O113 (6)	O121 (7)	O128 (2)	Total (82)
aadA1	20	6	2	—	4	3	8	2	6	—	51
TetA^{AB,c}	14	4	1	—	2	2	8	2	1	—	34
tetB	15	3	3	1	3	1	—	3	2	2	33
dfrA1	17	7	3	1	4	—	8	4	3	1	48
qnr	18	5	—	1	5	1	9	—	6	1	46
aac(3)-IV	8	3	2	—	—	—	4	2	5	—	24
sul1^{a}	29	6	3	1	2	—	4	5	3	—	53
BlaSHV^{a}	1	—	—	—	—	—	2	—	—	—	3
CITM	3	1	—	—	—	1	—	1	2	—	8
cat1	12	5	2	—	2	2	2	1	—	—	26
cmlA^{A}	14	2	—	—	1	—	—	2	5	—	24
TE30	30	6	4	1	4	2	8	4	4	—	63
S10	16	5	3	1	3	1	6	3	6	1	45
C30	30	7	3	—	5	3	4	1	7	—	60
SXT	25	4	2	1	2	—	3	2	3	2	44
GM10	10	5	4	—	—	2	7	4	2	—	34
NFX5	20	8	2	—	1	—	4	1	1	1	38
L2	16	6	1	—	3	3	6	—	—	—	35
CF30^{B,b}	2	1	—	—	—	—	3	—	—	—	6
CIP5	10	5	4	1	2	2	2	4	—	—	30
TMP5	15	6	4	—	3	1	7	5	6	1	48
F/M300^{b}	24	7	2	—	4	—	8	—	7	—	52
AM10^{c}	4	1	—	—	1	2	1	—	3	—	12

^{A,B}Dissimilar uppercase superscripts within a row indicate significant differences, $P < 0.01$.

$^{a–c}$Dissimilar lowercase superscripts within a row indicate significant differences, $P < 0.05$.

[1]Genes associated with resistance to streptomycin (*aadA1*), tetracycline (*tetA, tetB*), trimethoprim (*dfrA1*), quinolones (*qnr*), gentamicin [*aac(3)-IV*], sulfonamides (*sul1*), β-lactams (*blaSHV*, *CITM*), and chloramphenicol (*catA1, cmlA*), TE30 = tetracycline (30 μg/disk); S10 = streptomycin (10 μg/disk); C30 = chloramphenicol (30 μg/disk); SXT = sulfamethoxazol (25 μg/disk); GM10 = gentamycin (10 μg/disk); NFX5 = enrofloxacin (5 μg/disk); L2 = lincomycin (2 μg/disk); CF30 = cephalothin (30 μg/disk); CIP5 = ciprofloxacin (5 μg/disk); TMP5 = trimethoprim (5 μg/disk); F/M300 = nitrofurantoin (300 μg/disk); AM10 = ampicillin (10 μg /disk).

[2]Numbers in parentheses in the top row are numbers of positive samples in each serogroup.

Our results showed the high presence of serogroups, virulence factors, and multiple antibiotic-resistant properties of *E. coli* isolated from chicken meat samples. To the authors' knowledge, excessive prescribing, crowding, and poor sanitation are the primary factors responsible for the high antibiotic resistance in *E. coli* isolated from chicken meat.

In conclusion, the results of our study revealed that the PCR assay as an accurate, safe, and fast diagnostic method for detection of pathogens in meat samples. The veterinarians should pay more attention to antibiotic prescription. Results indicated that the *E. coli* virulence genes especially *stx1*, *stx2*, *eaeA*, and *ehly* are well distributed in poultry meat in investigated regions. Besides the O157 and O91 serogroups are the predominant serogroups of bacterium in chicken meat in Iran. Ahvaz city has a high temperature, and this city also had the highest prevalence of *E. coli* in poultry meat samples. Shahrekord city has a low temperature, so this city had the lowest prevalence of *E. coli* in poultry meat samples (*E. coli* is more active in warm places). It seems that sanitation conditions, especially in poultry slaughterhouses and supermarkets, help to reduce the contamination rate of poultry meat. In the current conditions in Iran, we suggest applying cephalothin and ampicillin for *E. coli*-infected cases. Finally, to prevent antibiotic resistance in bacteria, we have to prescribe antibiotics more cautiously in animals and periodically use different antibiotics, and be able to detect resistance genes and use disk diffusion methods.

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