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Incidence and Profiles of Antibiotic Resistance and Virulence Markers of The *Escherichia coli* O157 Bacteria Recovered From Poultry Meat

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Escherichia coli (*E. coli*) O157 issubstantial foodborne pathogen. The existing survey was addressed to assess the incidence, phenotypic resistance profile and incidence of virulence markers amongst the *E. coli* O157 isolates recovered from poultry meat. Five-hundred poultry meat samples were collected from Isfahan, Iran.Poultrymeatsamples were cultured and phenotypic antibiotic resistance pattern was deliberated using disk diffusion. PCR was applied to identify the incidence of virulence markers. Forty-four out of 500 (8.80%) poultry meat samples were contaminated with *E. coli* O157. Duck meat (16%) harbored the uppermost incidence of *E. coli* O157. Incidence of *ehlyA*, *stx2*, *stx1*and *eaeA* were 100%, 27.27%, 100% and 100%, respectively. *E. coli* O157 bacteria exhibited the maximum incidence of resistance against ampicillin (95.45%), tetracycline (88.63%), gentamycin (84.09%)and trimethoprim (38.63%) antibiotics. Incidence of resistance toward imipenem and chloramphenicol were 6.81% and 27.27%, respectively. Poultry meat samples, particularly duck, were considered as reservoir of virulence and antibiotic resistant O157 bacteria. Thoughtful antibiotic prescription and courtesies to the ideologies of food security can condense the hazard of resistant and virulent *E. coli* O157 in poultry meat.

Keywords: Incidence, *Escherichia coli* O157, Antibiotic resistance, Virulence markers, Poultry meat.

Introduction

Poultry meat is nutrient foodstuff with boost beneficial effects on human health. It is rich source of protein, fat and some kinds of vitamins essential for healthy life [1]. However, considering the low hygienic circumstances of abattoir, several outbreaks of foodborne diseases have been reported in diverse parts of the world [1,2].

Escherichia coli (*E. coli*) is significant cause of foodborne diseases [3]. Poultrymeatis such chief sources of *E. coli* [4]. Enterohemorrhagic *E. coli* (EHEC) bacteria are a dangerous phenomenon originated from Shiga toxin-producing *E. coli* (STEC) [3-5]. They are accountable for hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), hemorrhagic colitis (HC) and diarrhea [6, 7]. O157 is a chief serogroup of the above mentioned phenomena with boost clinical standing [3-7]. Poultry is one of the most significant sources of human infection [4].

E. coli O157 bacteria harbored the boost incidence of intimin (*eaeA*), Shiga toxins (*stx1* and *stx2*) and hemolysin (*hlyA*). They act as adhesive and invasive factors and are chiefly accountable for occurrence of attaching-effacing (A/E) lesions which mainly caused by the *eaeA*gene [3-7].

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These markers are chiefly accompanying with occurrence of clinical syndromes and are attended with bacterial adhesion and invasion to host cells.

E. coli O157 bacteria are chiefly resistant toward numerous kinds of antibiotics including aminoglycosides, fluoroquinolone, tetracyclines, sulfonamides, and phenicols [7-10]. *E. coli* O157 bacteria displayedboostincidence of resistance (50-100%) toward normally applied antibiotics [7-10]. Resistant *E. coli* O157 bacteria are chiefly hard to treat and cause severe clinical syndromes for longer time.

Numerous researches have been led on molecular epidemiology of *E. coli* O157 bacteria in food stuffs. Consequently, an existing examination was performed to measure the phenotypic profiles of antibiotic resistance and delivery of virulence factors amongst the *E. coli* O157 bacteria recovered from chicken, turkey, quail, duck and ostrichmeat samples in Iran.

Materials and Methods

Moral deliberation

The survey was allowed by the Moral Panel of the Islamic Azad University, Shahrekord Branch, Iran.

Samples

From April to August 2016, a total of 500poultry meats amples suchas chicken (n=100), turkey (n= 100), quail (n= 100), duck (n= 100) and ostrich (n= 100) meat samples were randomly collected from retail centers of the Isfahan province, Iran. The Isfahan province covers an area of roughly 107,027 square km and is situated in the center of Iran with 5,121 million populations. The external surfaces of poultry meat samples were disinfected with 70% alcohol in order to minimize cross contamination. The pieces of the muscles (100 g from the femur muscle) were collected separately into sterile bags using sterile scissors and tissue forceps. All samples were immediately transported to the laboratory (Food Hygiene Research Center, Shahrekord Branch, IAU, Iran) in cooled boxes.

E. coli O157 isolation and identification

Bacterial isolation was performed rendering the protocols labeled beforeh and [11,12]. For this goal, 25 g of samples were normalized well and one of the achieved solution was blended with 5 mL of buffered peptone water (Merck, Germany). Media were then incubated at 37 °C for 24 h. MacConkey sorbitol agar (Merck, Germany) was applied for determination of O157 sero group. Definitive detection of O157 serogroup was performed using the Latex agglutination examination in sorbitol negative bacteroid[11]. Diverse biochemical tests such as indole, methyl-red, Voges–Proskauer and citrate (IMVC) and Triple Sugar Iron Agar (TSIS) were also applied for identification of bacteria [12].

PCR detection of virulence factors

O157 isolates were cultured on nutrient broth (Merck, Germany) and further incubated at 37 °C for 24 h. Principles of producing factory of DNA extraction kit (Thermo Fisher Scientific, Germany) were applied for DNA extraction. Extracted DNA samples were subjected to quantification by NanoDrop device (NanoDrop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel) and purity checking (A260/A280). PCR has conducted rendering beforehand documents (Table 1) [5]. Thermo-cycler device (Flexrcycler^2, Germany) was used. Fifteen microliters of the PCR products were electrophoresed using 1.5% agarose gel (5). Runs were comprised a negative control (PCR grade water) and positive control (*E. coli* O157: K88ac:H19).

Antimicrobial susceptibility testing

Phenotypic profile of antibiotic resistance of O157 isolates were examined by disk diffusion test. Mueller–Hinton agar media (Merck, Germany) were applied for this goal. Protocols of the Clinical and Laboratory Standards Institute (CLSI) were applied for this goal [13]. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol was applied for this goal. Diverse antibiotic disks (Oxoid, UK) includingenrofloxac in (5 µg), imipenem (30 u), trimethoprim (5 µg), ampicillin (10 u), ciprofloxacin (5 µg), cefotaxime (30 µg), cotrimoxazole (30 µg), gentamycin (10 µg), sulfamethoxazole (25 µg), tetracycline (30 u), cefipime (30 µg), and chloramphenicol (30 µg) was applied for this goal (antibiotic was selected rendering their frequency of use in medicine and veterinary). An entire of 0.5 McFarl and concentrations of bacteria was applied for this goal.

Statistical examination

Data gotten from the experimentations were classified in the Excel software. SPSS/21.0 software was accompanied for statistical examination. Chi-square and Fisher's exact two-tailed tests were applied to measure any noteworthy association. Statistical signification was determined at a P value < 0.05.

Results

Table 2 epitomizes the incidence of *E. coli* O157 in diverse poultry meat samples. Forty-four out of 500 (8.80%) poultry meat samples were contaminated with *E. coli* O157 bacteria. Duck meat (16%) harbored the uppermost incidence of *E. coli* O157 bacteria, while quail meat (3%) had the lowermost. Statistical remarkable variance was gotten amid type of poultry meat samples and incidence of *E. coli* O157 ($P<0.05$).

Table 3 characterizes the distribution of virulence genes amongst the *E. coli* O157 bacteria isolated from poultry meat samples. *Stx1* (100%), *eaeA* (100%) and *ehlyA* (100%) were the most routinely identified virulence genes amongst the *E. coli* O157 bacteria isolated from poultry meat samples. Distribution of *stx2* virulence gene was 27.27%. *E. coli* O157 bacteria isolated from chicken meat samples had a higher distribution of *stx2* gene ($P<0.05$). All isolates were simultaneously positive for *stx1*, *eaeA* and *ehly* genes (100%).

E. coli O157 bacteria exhibited the maximum incidence of resistance against ampicillin (95.45%), tetracycline (88.63%), gentamycin (84.09%) and trimethoprim (38.63%) antibiotics. Distribution of resistance against imipenem (6.81%) and chloramphenicol (27.27%) was lower than other tested antibiotic agents. Statistical remarkable variance was gotten for the distribution of antibiotic resistance between different samples ($P<0.05$).

TABLE 1. The oligonucleotide primers and the PCR programs used for amplification of virulence factors of *E. coli* O157 isolates of poultry meat.

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR Volume (50µL)
stx1 (Shiga toxin I)	F: AAATCGCCATTCGTTGACTACTTCT R: TGCCATTCTGGCAACTCGCGATGCA	366	1 cycle: 95 ^{0C} ---------- 3 min. 34 cycle: 94 ^{0C} ---------- 60 s 56 ^{0C} ---------- 45 s 72 ^{0C} ---------- 60 s 1 cycle: 72 ^{0C} -------- 10 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
stx2 (Shiga toxin II)	F: CGATCGTCACTCACTGGTTTCATCA R: GGATATTCTCCCCACTCTGACACC	282		
EaeA (Intimin)	F: TGCGGCACAACAGGCGGCGA R: CGGTCGCCGCACCAGGATTC	629		
Ehly (Hemolysin)	F: CAATGCAGATGCAGATACCG R: CAGAGATGTCGTTGCAGCAG	432		

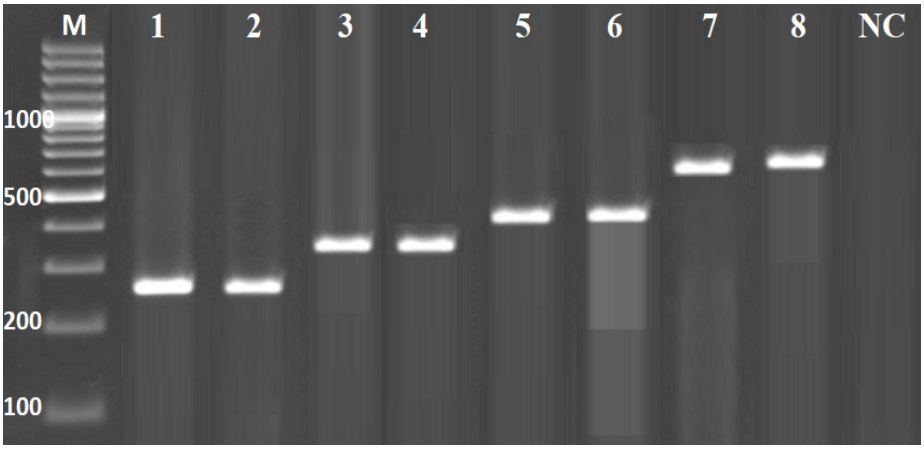

Fig. 1. PCR visualization of virulence factors. M: 100 bp ladder, Lane 1: Positive sample for *stx2* marker (282 bp), Lane 2: positive control for *stx2* marker, Lane 3: Positive sample for *stx1*marker (282 bp), Lane 4: positive control for *stx1* marker, Lane 5: Positive sample for *ehlyA* marker (282 bp), Lane 6: positive control for *ehlyA* marker, Lane 7: Positive sample for *eaeA* marker (282 bp), Lane 8: positive control for *eaeA* marker, NC: Negative control (PCR-grade water).

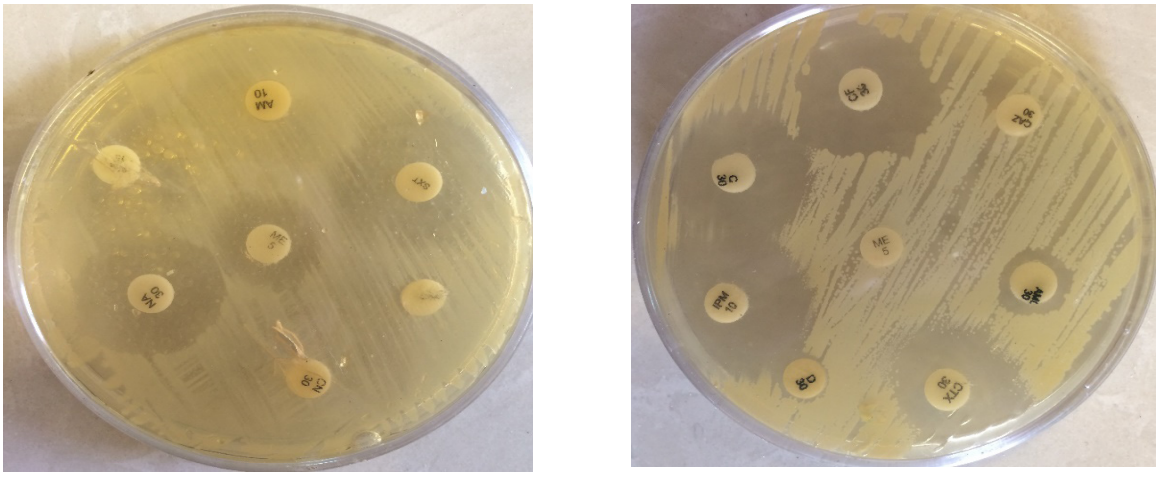

Fig. 2. Samples of antibiotic resistance patterns of *E. coli* O157 bacteria recovered from poultry meat.

TABLE 2. Incidence of *E.coli* O157 in poultry meat samples.

Types of samples	No. samples collected	No positive strains (%)
Chicken	100	12 (12)
Turkey	100	5 (5)
Quail	100	3 (3)
Ostrich	100	8 (8)
Duck	100	16 (16)
Total	500	44 (8.80)

TABLE 3. Distribution of virulence factors in *E. coli* O157 strains isolated from poultry meat samples.

Types of samples (No. *E. coli* O157 positive samples)	Distribution of virulence genes (%)				
	stx1	***stx2***	***eaeA***	***ehly***	***stx1+eaeA+ehly***
Chicken (12)	12 (100)	4 (33.33)	12 (100)	12 (100)	12 (100)
Turkey (5)	5 (100)	1 (20)	5 (100)	5 (100)	5 (100)
Quail (3)	3 (100)	1 (33.33)	3 (100)	3 (100)	3 (100)
Ostrich (8)	8 (100)	2 (25)	8 (100)	8 (100)	8 (100)
Duck (16)	16 (100)	4 (25)	16 (100)	16 (100)	16 (100)
Total (44)	44 (100)	12 (27.27)	44 (100)	44 (100)	44 (100)

TABLE 4. Antibiotic resistance pattern of *E. coli* O157 strains isolated from poultry meat samples.

Samples (N positive)	Antibiotic resistance pattern (%)									
	Tet*	Cfx	Gen	Cot	Enr	Cip	Imp	Amp	Tri	C30
Chicken (12)	10 (83.33)	5 (41.66)	10 (83.33)	4 (33.33)	6 (50)	5 (41.66)	1 (8.33)	11 (91.66)	4 (33.33)	4 (33.33)
Turkey (5)	4 (80)	1 (20)	4 (80)	2 (40)	2 (40)	2 (40)	-	5 (100)	2 (40)	1 (20)
Quail (3)	3 (100)	-	3 (100)	-	2 (66.66)	1 (33.33)	-	3 (100)	2 (66.66)	1 (33.33)
Ostrich (8)	7 (87.50)	3 (37.50)	6 (75)	3 (37.50)	4 (50)	3 (37.50)	-	8 (100)	3 (37.50)	2 (25)
Duck (16)	15 (93.75)	4 (25)	14 (87.50)	5 (31.25)	7 (43.75)	5 (31.25)	2 (12.50)	15 (93.75)	6 (37.50)	4 (25)
Total (44)	39 (88.63)	13 (29.54)	37 (84.09)	14 (31.81)	21 (47.72)	16 (36.36)	3 (6.81)	42 (95.45)	17 (38.63)	12 (27.27)

*Tet: tetracycline (30 u/disk), Cfx: cefotaxime (30 µg/disk), Gen: gentamycin (10 µg/disk), Cot: cotrimoxazole(30 µg/disk), Enr: enrofloxacin (5 µg/disk), Cip: ciprofloxacin (5 µg/disk), Imp: imipenem (30 u/disk), Amp: ampicillin (10 u/disk), Tri: trimethoprim (5 µg/disk), C30: chloramphenicol (30 µg/disk).

Discussion

E. coli O157 is measured as a hazardous cause of gastrointestinal disorders associated with consumption of foods with animal origin. Protagonist of poultry meatina broadcast of *E. coli* O157 to human has been documented in roughly literature works [14, 15].

An existing survey is one of the most inclusive reports of phenotypic description of antibiotic resistance and incidence of virulence factors in the *E. coli* O157isolates recovered from raw chicken, ostrich, quail, turkey and duck meat samples in Iran. Our findings recognized that 8.80% of poultry meat samples were contaminated with *E. coli* O157. As the *E. coli* O157 bacteria were isolated through the culture method, thus they were alive and had the ability of growth and invasion into the cells of their hosts. Thus, consumption of raw or undercooked chicken, ostrich, quail, turkey and duck meat samples may cause severe clinical syndromes such as HUS, TTP, HC and diarrhea. Duck meat samples had the uppermost incidence of *E. coli* O157 bacteria. Dissimilar diet and living in humid environments are probable reasons for higher incidence of bacteria in duck meat. Likelihood of cross contamination occurrence in the abattoirs amid poultry meat carcasses and feces is another imperative reason for the boost incidence of O157 bacteria in poultry meat samples. Diverse surveys have been conducted in a similar topic to our research. Awadallah et al. [16] disclosed that the incidence of *E. coli* in quail meat was 47% which was higher than our discoveries. Lyhs et al. [17] conveyed that the incidence of *E. coli* in poultry meat was 94.50%. Xia et al. [18] conveyed the higher incidence (23.50%) of *E. coli*in turkey meat samples. Furthermore, boost incidence of *E. coli* O157 in meat samples was reported by Hossain et al. [19] (Saudi Arabia) (2-10%), De Giusti et al. [20] (Italy) (2.61%), Momtaz et al. [5] (Iran) (25-36%) and Ranjbar et al. [3] (Iran) (25-34%). The incidence of *E. coli* O157 in meat samples demonstrated to be changeable in various areas owing to variation in number of livestock, season of sampling, hygienic circumstances in each farm, levels of farm management, sampling oddness, discrepancy in kind of samples, and departure in methods of pathogenic detection.

Our discoveries signified that O157 bacteria harbored both virulence markers and also resistance toward generally applied antibiotic agents. This matter may disclose boost pathogenicity of O157 isolates. Furthermore, it can highlight the role of poultry meat in transmission of antibiotic resistance to human population. Presence of virulent and antibiotic resistant O157 bacteria in diverse kinds of food samples, particularly ruminants and poultry meat and their products have also been conveyed in different surveys [1-10, 12]. Concurrent atten dance of *ehlyA*, *stx2*, *stx1*, and *eaeA*virulence markers have also been conveyed in the *E. coli* bacteria isolated from diverse kinds of food samples in Nigeria [21], United States [22] and Iraq [23]. Majority of *E. coli* O157 isolates were resisting toward ampicillin, tetracyclines, gentamicin andtrimethoprim antibiotic agents. Unlawful and imprecise antibiotic prescription particularly in poultry fields is may be the chief reason for the boost incidence of resistance in the *E. coli* O157. Boost incidence of resistance of *E. coli* bacteria toward ampicillin, tetracyclines, gentamicin and trimethoprim antibiotic agents was also conveyed from the United States [24], Estonia [25] and Saudi Arabia [26]. Recent work[5] conveyed that O157 isolates recovered from meat samples harbored boost phenotypic antibiotic resistance toward penicillin (100%), tetracycline (80.59%), gentamicin (55.22%) and trimethoprim (40.29%) antibiotics. Another survey[1] also conveyed that the incidence of resistance towardtetracycline, ampicillin, gentamicin, ciprofloxacin and penicillin antibiotics were 93.33%, 91.11%, 51.11%, 68.88% and 68.88%, respectively. Previous investigation[14] conveyed that *E. coli* O157 isolates recovered from chicken meat samples displayed the uppermost incidence of resistance toward tetracycline (96.77%), chloramphenicol (96.77%), and sulfamethoxazole (80.64%). Boost incidence of resistance toward chloramphenicol is mostly owing to an excessive application of this forbidden antibioticin poultry fields. Boost incidence of resistance toward chloramphenicol was also conveyed in investigations conducted on the United States [27], Ethiopia [28] and Iran [29]. Similar phenotypic profiles of antibiotic resistance were also conveyed from South Africa [30] (boost incidence of ampicillin and tetracycline), India [31] (boost incidence of resistance toward gentamicin, cephalothin, erythromycin, kanamycin andamikacin), Mexico [32] (boost incidence of resistance toward

ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole and cephalothin) and South Korea [33] (boost incidence of resistance toward streptomycin, ampicillin, amikacin and tetracycline). Recent report[34] testified that the incidence of *E. coli* in raw meatwas 14%. She disclosed that O157 bacteria harbored concurrent presence of *stx1*, *eaeA* and *ehly* virulence markers. She also disclosed that incidence of resistance of O157 bacteria toward gentamycin, ciprofloxacin, tetracycline and ampicillin was 90.47%, 71.42%, 85.71% and 100, respectively. Absolutely, percentage of food-borne microbes, predominantly bacteria, in occurrence of food-borne clinical syndromes has been measured in Iran and diverse surveys have been conducted in this field [35-44].

Conclusions

In deductions, boost incidence of *E. coli* O157 bacteria was perceived in chicken, duck, turkey, ostrich and quail meat samples. Furthermore, boost incidence of resistance toward generally used antibiotics and imperative incidence of *stx1*, *eaeA* and *ehlyA* virulence markers were also perceived. Concurrent presence of more than one virulence markers disclose boost pathogenicity of O157 strains. Additionally, poultry meat samples were considered as source of pathogenic *E.coli* O157 bacteria and also vehicle for transmission of antibiotic resistance to human population. Our survey emphasized an imperative epidemiological hazard regarding the consumption of raw poultry meat samples. By means of an appropriate thermal dispensation and suitable inspection can lessen the hazard of *E. coli* O157 in poultry meat. Nevertheless, supplementary explorations are obligatory to determine supplementary epidemiological and microbiological features of *E. coli* O157 in poultry meat.

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Conflict of interest

The author has no conflict of interests to declare regarding the publication of this paper.

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