Prevalence of Shiga toxin-producing and Enteropathogenic Escherichia coli Isolated from Chicken Meat in the west of Iran

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Research note

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Abstract

Objective: Shiga toxin-producing Escherichia coli (STEC) is known as a crucial zoonotic foodborne pathogen. Totally, 257 raw chicken meat were collected from markets in Hamadan, west of Iran. The samples were cultured on selective media and the virulence genes of E. coli isolates were analyzed by PCR. The antibiotic resistance patterns were determined by the disk diffusion method. The genetic relatedness of the E. coli O157 isolates was analyzed by ERIC-PCR. Results: Totally, 93 (36%; 95% CI 41.9-30.1%) isolates were identified as E. coli. Based on microbiological tests, 36 (38.7%; 95% CI 48.6-28.8), 7 (7.5%; 95% CI 12.8-2.2%), and 12 (12.9%; 95% CI 19.7- 6.1%) of the E. coli isolates were characterized as STEC, Enteropathogenic E. coli, and attaching and effacing E. coli (AEEC) strains, respectively. A high level of resistance to nalidixic acid (91.4%; 95% CI 97.1- 85.7%), tetracycline (89.8%; 95% CI 96.2-83.5%), ampicillin (82.8%; 95% CI 90.2-75.1%), and sulfametoxazole-trimotoprime (71%; 95% CI 80.2-61.8%) was detected among the E. coli isolates. The analysis of ERIC-PCR results showed five different ERIC types among the E. coli O157 isolates. Based on findings. Control and check-up of poultry meats should be considered as a crucial issue for public health.

Introduction

Diarrheagenic *E. coli*, which causes diarrhea in humans, can be classified into seven different pathotypes on the basis of its specific virulence properties, distinct epidemiology, and clinical features: Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffuse-Adhering *E. coli* (DAEC), Cytolethal distending toxin-producing *E. coli*, Enteropathogenic *E. coli* (EPEC), and Shiga toxin-producing *E. coli* (STEC) [1]. STECs are one of the most important pathogens transmitted by food. In addition to causing food poisoning, these strains can cause severe diseases such as diarrhea, bleeding colitis, hemolytic uremic syndrome, thrombocytopenic purpura, and death. Most cases of ulcerative colitis and hemolytic uremic syndrome are related to the O157:H7 serotype which is considered as the most important serotype of this strain. Several outbreaks of bacterial foodborne disease due to the consumption of undercooked or raw meat contaminated with STEC strains have been reported [1, 2].

In addition to Shiga toxins, an external membrane protein called intimin is responsible for the attachment of bacteria to the intestinal epithelial cells, causes a certain damage (attaching-effacing lesions (A/E)), and is encoded by the *eae* gene [3, 4]. Also, enterohemolysin, encoded by the *hly* gene, is an effective factor in the pathogenicity of STEC [5]. Because only limited and incomplete studies have been conducted on the prevalence and epidemiology of the O157:H7 serotype in developing countries, its prevalence has been reported as low [6, 7].

The EPEC pathovar plays an important role as a causative agent of infantile diarrhea in developing countries [8]. This pathovar has intimin which is encoded by the chromosomal *eae* gene. It also possesses the ability to form A/E lesions on intestinal cells but does not contain Shiga toxin-encoding genes.

Attaching and effacing *E. coli* (AEEC) are characterized by their ability to cause attaching and effacing (A/E) lesions in the gut mucosa of human and animal hosts leading to diarrhea. Thus, two groups of *E. coli* strains that cause attaching and effacing (A/E) lesions are classified as AEEC. In Iran, most molecular studies on the STEC have been done on dairy and animal stool samples and little information is available on STEC and EPEC strains from poultry sources. The aim of this study was to detect the virulence factors *stx1*, *stx2*, *eae,* and *hlyA* in the *E. coli* isolates and also to perform molecular typing of O157:H7 strains isolated from raw chicken meat samples.

Methods

Identification of *E. coli* strains

In this cross-sectional study, 257 raw chicken meat samples were randomly collected using an electronic random number generator (www.randomresult.com) from different butchers and supermarkets of different area of Hamadan city, west of Iran, from January 2016 to May 2017. The samples were transferred to sterile tubes containing thioglycolate broth media after homogenization, and incubated overnight at 37 ºC. They were inoculated on MacConkey agar plates (Merck, Germany) and incubated at 37°C for 24 h. The *E. coli*-like colonies were subjected to different biochemical tests including sugar fermentation, Simmons' citrate, indole production, motility, methyl red, and Voges-Proskauer (IMVIC) tests [1]. The sorbitol MacConkey agar (Merck, Germany) and serogrouping with anti-O157 sera (Baharafshan, Iran) were used for the diagnosis of the *E. coli* O157 serotype.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of *E. coli* isolates to cefotaxime (CTX), ceftazidime (CAZ), cefepime (CPM), cefoxitin (FOX), cefexime (CFM), nitrofurantoin (NIT), gentamicin (GEM), ciprofloxacin (CIP), nalidixic acid (NA), sulfamethoxazole-trimethoprim (SXT), aztreonam (ATM), amoxicillin (AMX), ampicillin (AMP), tetracycline (TET), minocycline (MIN), and imipenem (IPM) was detected by the disk diffusion method according to CLSI guidelines [9].

Detection of virulence genes

A sweep of five *E. coli* colonies on MacConkey agar was inoculated in LB broth and incubated overnight at 37 °C and the genomic DNAs of the colonies were extracted by boiling method [10]. The virulence genes s*tx1, stx2, hlyA,* and *eae* were detected by PCR method using the primers described in previous studies [11].

The virulence genes *eae*, *stx1*, and *stx2* were detected using a triplex PCR in a reaction mixture with a total volume of 20 µL, included 10 µL PCR Master Mix 2x (Fermentas, Lithuania), 6 µL double distilled water, 1 µL from each primers, and 2 µL DNA template. The cycling program was used as follows: initial denaturation (3 min at 94 °C), followed by 35 cycles of denaturation (1 min at 94 °C), annealing (1 min at 55 °C), extension (1 min at 72 °C), and final extension (7 min at 72 °C). For the *hly* gene, a single PCR

reaction was done with the same conditions as mentioned above except that annealing was at 63 °C for 1 min.

ERIC-PCR of *E. coli* O157 isolates

For molecular typing and detection of the genetic linkage among *E. coli* O157 serotypes, ERIC-PCR was carried out using ERIC primers and the conditions described in a previous study [12]. The banding patterns of ERIC were analyzed by an online data analysis service (inslico.ehu.es). The ERIC profiles were compared by the Dice method and were clustered by the UPGMA program.

Results And Discussion

Among the 257 raw poultry samples, 93 (36%; 95% CI 41.9- 30.1%) isolates were identified as *E. coli*. Based on serological and microbiological tests, 36 (38.7%; 95% CI 48.6-28.8), 7 (7.5%; 95% CI 12.8-2.2%), and 12 (12.9%; 95% CI 19.7- 6.1%) *E. coli* isolates were characterized as STEC (*stx*1$^+$ and/or *stx*2$^+$ and *eae+*/*eae*$^-$), EPEC (*eae*$^+$), and AEEC strains (EPECs and *eae+* strains of STECs), respectively. All of the STEC isolates showed colorless colonies on the sorbitol MacConkey media.

The results of the antimicrobial susceptibility test conducted on 93 *E. coli* isolates are shown in Figure 1. Based on the results, all of the isolates (100%) were susceptible to cefotaxime, cefoxitin, ceftazidime, and aztreonam. A high-level of resistance to nalidixic acid (91.4%; 95% CI 97.1- 85.7%), tetracycline (89.8%; 95% CI 96.2-83.5%), ampicillin (82.8%; 95% CI 90.2-75.1%), and sulfamethoxazole-trimethoprim (71%; 95% CI 80.2-61.8%) was detected among the *E. coli* isolates. The PCR results showed that the distribution of the virulence genes *stx*1, *stx*2, and *eae* among the 93 *E. coli* isolates was 15 (16.1%; 95% CI 23.6-8.6%), 31 (33.3%; 95% CI 42.9-23.7%), and 12 (12.9%; 95% CI 19.7-6.1%), respectively. All of *E. coli* O157 strains showed *stx1*$^+$/*stx2*$^+$/*eae*$^+$ (1 isolate), *stx1*$^+$/*eae*$^+$ (2 isolates), and *stx2*$^+$/*eae*$^+$ (2 isolates) patterns. The *hly*A gene was not detected in any of the *E. coli* isolates (Figure 2). The analysis of the ERIC-PCR results showed genetic diversity among *E. coli* O157 strains because five different ERIC patterns were observed among these strains (Figure 3).

The results shown that chicken meat can be contaminated with *E. coli*. This organism was isolated from 93 (36%) raw chicken meat samples and 36 (38.7%), 7 (7.5%), and 12 (12.9%) of the *E. coli* isolates were characterized as STEC, EPEC, and AEEC strains. The *stx*2 gene was the most frequent virulence factor among the STEC isolates. The major animal source of STEC is primarily cattle, followed by sheep, goats, pigs, and poultry. Poultry meat is known as the potential source of STEC contamination compared to other sources of meat. In Korea, STEC was isolated in 22.6% of beef, 7.3% of poultry, and 2.0% of pork meat samples [13]. In the current study, O157 *E. coli* isolates having *stx1* and/or *stx2* and *eae* were detected in 5.3% of the poultry meat samples and recognized as STEC strains. Although the prevalence of this isolate was not significant, this rate of infection is considerable from the public health point of view.

The prevalence of STEC and AEEC in the current study is different from that of some studies in Iran and other countries. In the current study, higher STEC and lower AEEC isolates were detected compared to the

study of Momtaz el al. They reported that the prevalence of STEC and AEEC were 21% and 34%, respectively [14]. They also reported that *stx1* was the most frequent (96%) virulence factor among the isolates. In contrast, in the current study, *stx1* was found only in 16% of the isolates. One of the reasons for this difference in frequency can be the difference in the number of samples studied. However, Guran et al. showed that the overall prevalence of *E. coli* O157 in poultry meat samples collected from supermarkets in Diyarbakir, Turkey was 1.3% [15]. One of the significant results of the current study is that 12.9% of the *E. coli* isolates were identified as AEEC. Intimin genes are present in EPEC and in some STEC. Atypical EPEC or AEEC appears to be more closely related to STEC [15-17]. Based on the results of the current study, the role of AEEC strains in gastrointestinal infection needs further investigations.

In this study, the *E. coli* O157 strains were positive for *stx1, stx2,* and *eae* genes. In India, Dutta et al. reported that 14 (33.33%) isolates carried at least 1 virulence genes and 10 (23.81%) of these isolates (collected from poultry samples) were recorded as STEC and 4 (9.52%) of them were recorded as EPEC [18].

In a review study, the resistance rates of *E. coli* strains to tetracycline, sulfamethoxazole, streptomycin, and ampicillin were more than 40% in all the studied countries. Increasing antibiotic resistance is a major concern for animal and human health because of the high consumption of antibiotics in veterinary medicine. Resistant bacteria can spread from food-producing animals to humans. The information from the evaluated countries indicates that such antibiotics are usually used in poultry industry [19].

In this study, the resistance levels of STEC to some antimicrobial agents such as nalidixic acid, ampicillin, tetracycline, and trimethoprim-sulfamethoxazole ranged from 71 to 91%. According to these results, the poultry meat contaminated with STEC strains can be a potential source of antimicrobial resistance.

Momtaz et al. reported the high resistance of STEC strains to tetracycline, chloramphenicol, and nitrofurantoin (63 to 77%). According to our findings and studies by others, the prescription of tetracycline is recommended neither in cases of *E. coli* infection nor in veterinary medicine with respect to poultry products [14]. There are few reports about the molecular typing of STECs from poultry sources in Iran and other countries. In the current study, ERIC-PCR genotyping demonstrated 5 different ERIC-genotypes from 5 *E. coli* O157 isolates. Therefore, the results of the current study showed genetic diversity among *E. coli* O157 isolates as well as the different potential sources of *E. coli* O157 contamination. The results also indicated the usefulness of the PCR-based genotyping method in the epidemiological investigations of virulent *E. coli* strains. Consistent with our results, in a study by Sekhar et al. in India, the ERIC-PCR results discriminated 12 STEC isolates from poultry samples into 11 ERIC-PCR genotypes [20].

In conclusion, the results of the current study revealed that poultry meat can be considered as a source of pathogenic *E. coli* strains. Pathogenic *E. coli* strains in poultry meat samples were detected by such accurate and quick techniques as PCR assay. The detection of STEC (38%) was a significant finding. The *stx*2 was identified as the most frequent virulence factor among the STEC isolates. Our results indicate the need for more attention to poultry meat control, antibiotic administration in veterinarians and *E. coli*

virulence genes, especially *stx1, stx2 and eae*, which are largely present in pathogenic *E. coli* strains isolated from poultry meat.

Limitations

One of the most important limitations of this study was the rather few number of raw poultry meat samples. More samples are required for such molecular studies. We also had some limitations in financial support for obtaining information about poultry raising systems and slaughter systems to discuss the sources of contamination by robust typing methods.

Abbreviations

E. coli: Escherichia coli

STEC: Shiga toxin-producing *Escherichia coli*

AEEC: Attaching and effacing *Escherichia coli*

EPEC: Enteropathogenic *Escherichia coli*

Eae: Escherichia coli attaching and effacing

*stx*1 and *stx*2: Shiga toxin 1 and 2.

hly: Hemolysin

PCR: Polymerase chain reaction

Declarations

Authors' contributions

OZ, HH, and MA conceived the study. OZ and LS conducted the experiments and analyzed the results. OZ and LS drafted the manuscript and made substantial contributions to the design of the study. OZ, MA, and LS critically reviewed the manuscript. OZ and LS participated in data analysis. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All the information supporting our conclusions and appropriate references are included in the manuscript.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The present study was ethically approved by the Institutional Review Board of Hamadan University of Medical Sciences (IR.UMSHA.REC.1398.12).

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Figures

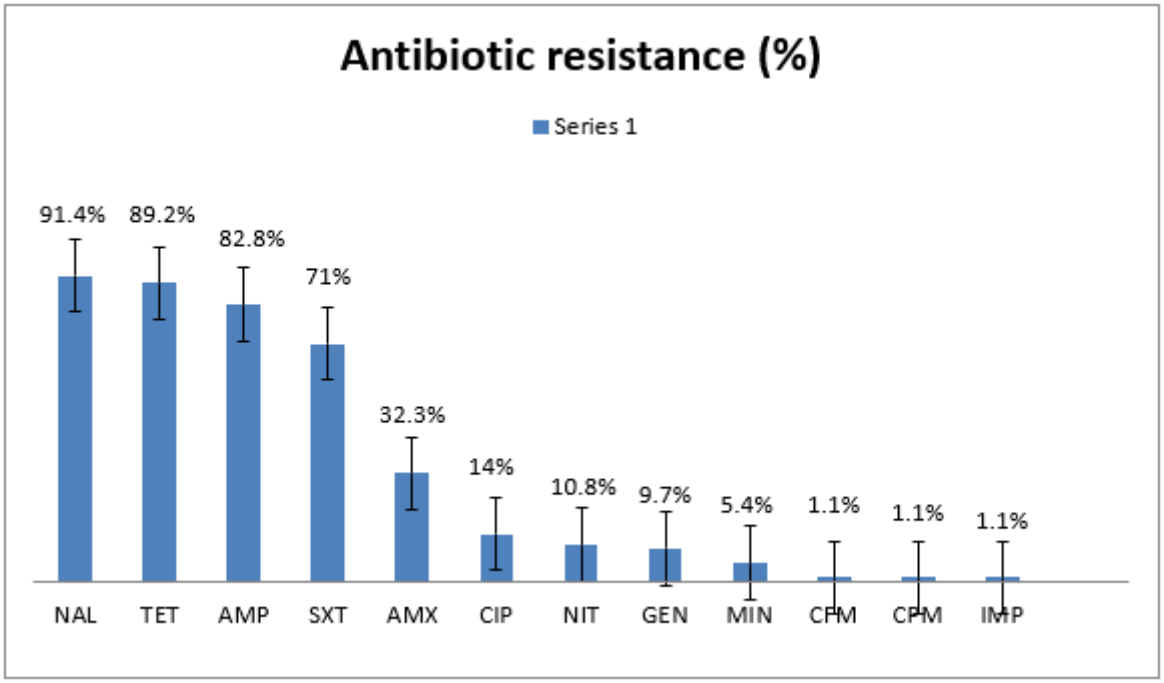

Figure 1

Antibiotic resistance (%) of E. coli isolated from raw chicken meats NA: nalidixic acid, TET: tetracycline, AMP: ampicillin, SXT: sulfamethoxazole-trimethoprim, AMX: amoxicillin, CIP: ciprofloxacin, NIT: nitrofurantoin, GEM: gentamicin, MIN: minocycline, CFM: cefexime, CPM: cefepime, IPM: imipenem.

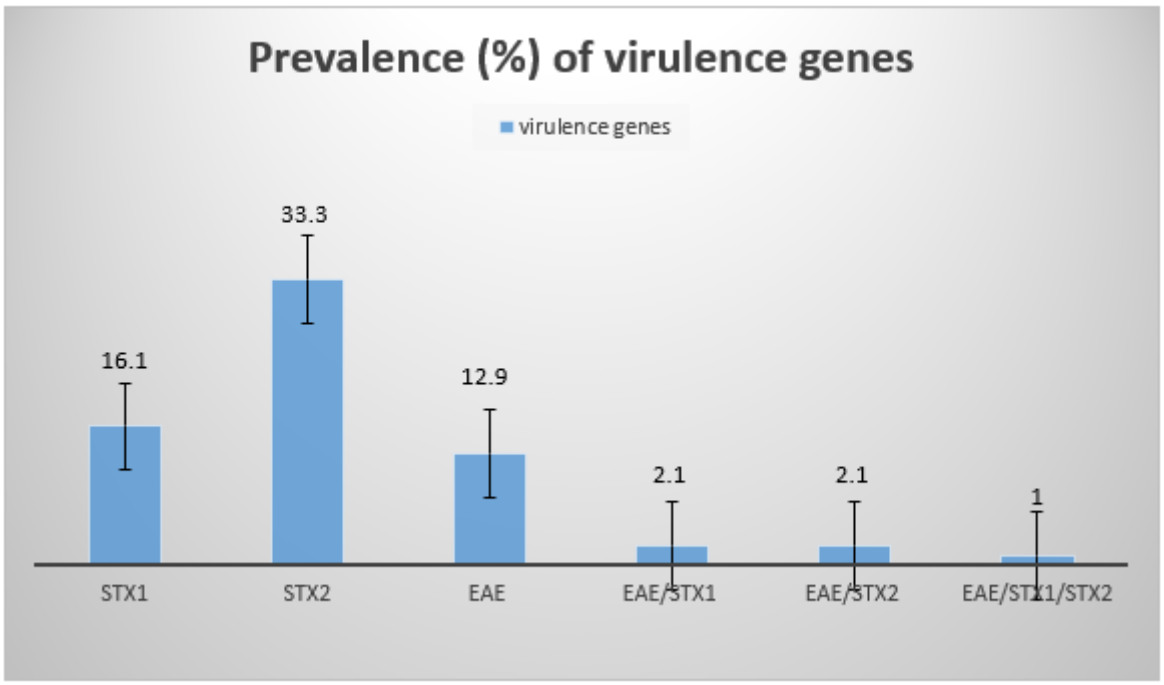

Figure 2

prevalence of virulence genes among 93 E. coli strains isolated from raw meat chicken

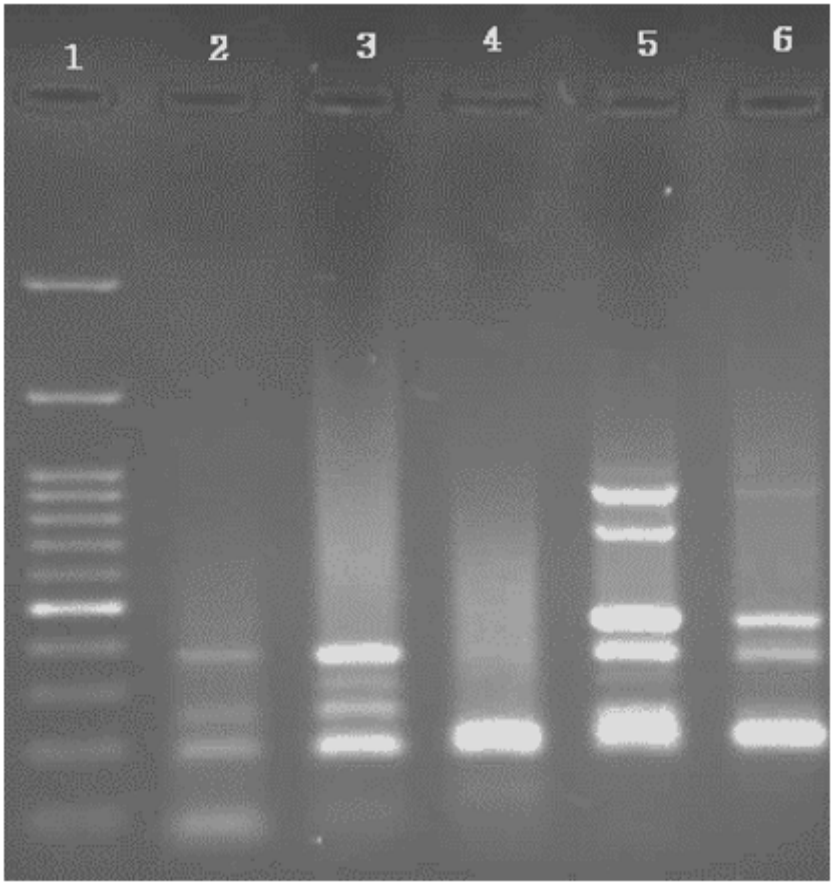

Figure 3

ERIC patterns of 5 different E. coli O157 isolates Lane 1: ladder 100 bp, lane 2-6: E. coli O157