Prevalence of Shiga toxin-producing Escherichia coli isolated from chicken meat in west of Iran

omid zarei
Tehran University of Medical Sciences
Leili Shokoohizadeh (Leilishokoohizadeh@gmail.com)
Hadi Hossainpour
Hamadan University of Medical Sciences Medical School
Mohammad Yousef Alikhani
Hamadan University of Medical Sciences Medical School https://orcid.org/0000-0003-4577-4029

Research note

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Abstract

Objective: Shiga toxin producing Escherichia coli (STEC) has known as a crucial zoonotic food borne pathogen. A total of 257 row chicken meat samples were collected from different markets in Hamadan city from January 2016 to May 2017. Samples were cultured on selective and differential culture media, and the virulence genes of E. coli isolates were analyzed by PCR assay. The antibiotic resistance patterns of E. coli isolates were determined by disk diffusion method. The genetic relatedness of STEC isolates were analyzed by ERIC-PCR. Results: Totally, 93(36%) of isolates were identified as E. coli in this current study. According serological and microbiological tests, 5(5.3%), 31(33.3%) and 7(7.5%) of E. coli isolates, characterized as Enterohemorrhagic E. coli (EHEC), STEC and attaching and effacing E. coli (AEEC) strains, respectively. High level resistance to tetracycline (89.8), ampicillin (82.8%) and sulfametoxazole-trimotoprime (71%) were detected among E. coli isolates. Analysis of ERIC-PCR results showed five different ERIC types among EHEC isolates. Based on our findings, chicken meat identified as a sources of STEC strains, therefore, the controlling and checkup the chicken meats for the resistance and virulent strains of E. coli should be consider as a crucial issues in public health.

Background

Enterobacteriaceae family bacteria is one of the most common gram-negative bacilli, some of which are normal flora and some are pathogenic. Shiga toxin producing *Escherichia coli* (*STEC*) are one of the most important pathogens transmitted by food. These strains, in addition to causing food poisoning, can cause severe diseases such as diarrhea, bleeding colitis, hemolytic uremic syndrome, thrombocytopenic purpura, and death. Most cases of hemolytic uremic ulcerative colitis and hemolytic uremic syndrome are related to 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 has been associated with STEC strains are one of the most important bacterial pathogen transmitted by food origin meat [1, 2].

In addition to shiga toxin, an external membrane protein called Intimin is responsible for the close connection of the bacteria to the intestinal epithelial cells and causes certain damage, called "effacing/attaching," and is coded by the *eae* gene [3, 4]. Also, the enterohemolysin, coded by the *hly* gene, is an effective factor in the pathogenicity of this serotype [5]. In developing countries, due to incomplete and limited studies on the prevalence and epidemiology of this serotype, the prevalence of O157:H7 is lower [6]. In Iran, most molecular studies on this bacterium relate to dairy and animal stool samples and inadequate evidence is available on STEC strains in poultry sources. Therefore, the aim of this study was to detect virulence factors *stx1*, *stx2*, *eaeA,* and *hlyA* in *E.coli* isolates and also molecular typing of these strains isolated from row chicken meat samples.

Methods

Identification of *Escherichia coli* strains

In the current study, a total of 275 row chicken meat samples were randomly collected from the butchers and market of different parts of Hamadan city, west of Iran, from January 2016 to May 2017. The meat samples transferred to sterile tubes and after homogenization, transported to tubes containing thioglycolate and incubated overnight at 35 ºC. *E. coli* strains were isolated according to conventional biochemical and microbiologic tests, including phenotype characterization using sorbitol-MacConkey agar and serotyping was performed using anti-O157 sera (Baharafshan, Iran) based on agglutination reaction assay.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *E. coli* strain to gentamicin (GM), ciprofloxacin (CIP), sulfamethoxazole-trimethoprim (SXT), aztreonam (ATM), amoxicillin (A), imipenem (IPM), nalidixic acid (NA), minocycline (MN), nitrofurantoin (FM) were detected by disk diffusion method according to CLSI guidelines in 2018.

Molecular detection of virulence genes

Genomic DNAs were extracted by boiling method. The virulence genes; s*tx1, stx2, hlyA,* and *eaeA* were detected by PCR using primers described previously [7].

DNA extraction and multiplex PCR assay for *stx1*, *stx2*, *eaeA*, *hlyA* genes amplification were performed as previously described by Kim et al [8]. The virulence genes *eae*, *stx1* and *stx2* were detected by a triplex PCR reaction by the following the program; initial denaturation (3 min at 94°C), followed by 35 cycles of denaturation (1 min at 94°C), annealing (1 min at 55 °C) and extension (1 min at 72°C) final extension at 72°C for 7 min and *hly* gene by a separate PCR reaction as mentioned above but annealing at 63°C for 1 min.

ERIC-PCR of EHEC strains

For molecular typing and detection of genetic linkage among EHEC strains, ERIC-PCR was carried out using the ERIC primers and condition that described previously by others [9]. The banding patterns of ERIC were analyzed by online data analysis service (inslico.ehu.es). ERIC profiles were compared using Dice method and clustered by UPGMA program.

Results And Discussion

Among 257 row chicken samples, 93(36%) isolates were identified as *E. coli*. According to serological and microbiological tests 5(5.3%), 31(33.3%) and 7 (7.5%) of *E. coli* isolates, characterized as Enterohemorrhagic *E. coli* (EHEC; positive for *stx*1 and/or *stx*2 and *eae*), STEC (positive for *stx1* and/or *stx*2), and attaching and effacing *E. coli (*AEEC*;* positive for *eaeA*) strains, respectively. All of the STEC isolates showed colorless colonies on MAC-sorbitol media culture.

The results of antimicrobial susceptibility test of 93 *E. coli* isolates shows in figure 1. According to antimicrobial susceptibility results, the resistance to antibiotics was not high in *E. coli* isolates. All of the

isolates were susceptible to cefotaxime, aztreonam, ceftazidime, and nitrofurantoin. Results showed high-level resistance to ampicillin (82.8%) and sulfamethoxazole-trimethoprim (71%). Resistance to imipenem, cefepime, and cefexime were detected only in 1.1% of isolates. Also, any resistance to antibiotics CTX, ATM and CAZ were not observed. According to PCR results, the distribution of virulence genes; *stx*1, *stx*2 and *eae* among 93 *E. coli* isolates were 15(16.1%), 31(33.3%) and 12(12.9%), respectively. All of EHEC isolates*, stx1/stx2/eae* (1 isolates), *stx1/eae* (2 isolates) and *sx2/eae* (2 isolates) patterns. The *hly*A gene was not detected in any of *E. coli* isolates (Figure 2). Analysis of ERIC-PCR results showed genetic diversity among EHEC isolates as well as five different ERIC patterns were observed among five EHEC isolates (Figure 3).

The results of our study have showed chicken meat can be contaminated by *E. coli,* this organism was isolated from (36%) of chicken row meat samples and 5(5.3%), 31(33.3%) and 7 (7.5%) of *E. coli* isolates, characterized EHEC, STEC, and AEEC strains. The sxt2 was more frequent virulence factor among STEC isolates. The major animal reservoirs of STEC are primarily cattle, followed by sheep, goats, pigs, and chicken. The chicken meat was known as the potential source of STEC as well as EHEC contamination after other sources. In Korea, STEC ,as well as EHEC, was isolated in 22.6% of beef, 7.3% of poultry, and 2.0% of pork meat samples [10]. In this current study, O157 *E. coli* isolates harboring *stx1* and or/ *stx2* and *eae* was detected in 5.3% of the chicken meat and recognized as EHEC. However, the prevalence of this isolates was not significant but this rate of infection is considerable from the public health point of view.

The rate of EHEC and AEEC is different from some studies in Iran and other countries. in our study, we have detected lower EHEC and lower AEEC isolates than which, Momtaz el al, have found, they have reported prevalence of EHEC and AEEC were 21% and 34% among 422 row chicken meat samples in different townships in Iran [11], they also reported *stx1* as more frequent (96%) virulence factor among isolates , however we found *stx1* in 16% of isolates. One of the reasons for the difference in frequency can be due to the difference in the number of samples studied. However, Guran et al, showed the overall *E. coli* O157 prevalence in chicken meat collected from supermarkets in Diyarbakir, Turkey was 1.3%, and 1.6% in China according to our results, all of the *E. coli* O157 isolates carried *eae**A* genes; but not any *hlyA* gene [12]. One of the significant results from this study are 12.9% of *E. coli* isolates was known as AEEC. “Attaching and effacing *Escherichia coli* (AEEC) are the cause of Attaching-and-Effacing (A/E) lesions in the gut mucosa of human and animal hosts leading to diarrhea. The adherence of bacteria to the enterocytes is mediated by Intimin, an outer membrane protein encoded by the *eae* (*E. coli* attachment effacement) gene Intimin genes are present in and some Shiga toxin-producing *E. coli* (STEC). Atypical enteropathogenic *E. coli* (EPEC) or AEEC appears to be more closely related to STEC” [13-15]. According to the detection of these isolates from chicken samples, the role of these strains in gastrointestinal infection needs more investigations [16].

In this study, the EHEC isolates were positive for *stx1, stx2,* and *eaeA* genes and also positive for O157 serotypes. In India, Dutta et al have reported 14 (33.33%) isolates carried at least 1 virulence gene, of which 10 (23.81%) and 4 (9.52%) were recorded as STEC and EPEC, from poultry birds [11, 17].

Gastrointestinal infections due to *E. coli* is one of the bacterial infections which need to treatment by antibiotics. According to a review study done by Roth et al, The resistance rates in *E. coli* isolated from broiler to tetracycline, sulfamethoxazole, streptomycin, and ampicillin, are higher than 40% in all countries. Increasing antibiotic resistance is a major concern for animal and human health. Because of the high consumption of antibiotics were observed, in veterinary medicine. Resistant bacteria can spread between food-producing animals and humans. According to reports from evaluated countries, Antibiotic such as tetracyclines, aminoglycosides, sulfonamides, and penicillins are used in poultry [18].

In this study, the high levels of resistance to some antimicrobial agents such as ampicillin, tetracycline, and trimethoprim-sulfamethoxazole, were 71 to 89%. According to these results, the STEC strains poultry meat origin are a potential reservoir of antimicrobial resistance. Accordance our results, in a study performed in Thailand 100% of *E. coli* isolates showed resistance to tetracycline, ampicillin, and erythromycin,

Momtaz et al have reported high resistant to tetracycline, chloramphenicol, and nitrofurantoin (63 to 77%). According to our findings and studies by others, Prescription of tetracycline is not recommended in cases of *E. coli* infection as well as in veterinary medicine in poultry[11]. There are rare reports based Molecular typing of STECs from Poultry sources in Iran and other countries. In this current study, ERIC-PCR genotyping demonstrated 5 different ERIC-genotypes from 5 STEC isolates .therefore, our results confirmed genetic diversity among EHEC isolates and also, potential different sources of EHEC contamination. Our results indicated to the utility of PCR-based genotyping method in the epidemiological investigations of virulent *E. coli* strains as well as EHEC strains. There is some limitation in this study because of the lack of human samples sources. However finding the related human sources samples is so difficult, unless in outbreaks episodes. Consistent to our results, in a study by Shekar et al, in India, ERIC-PCR results allowed discrimination of 12 STEC isolates from poultry samples into 11 ERIC-PCR genotypes [19]. Another limitation is insufficient number of samples. For better conclusion we need more sampling from different places in city in different intervals.

In conclusion, the results of our study revealed that poultry meat can be considered as a reservoir of pathogenic *E. coli* strains. We have detected pathogenic *E. coli* strains by accurate and quick techniques like PCR assay in chicken meat samples. Detection EHEC (5%) and STEC (33%) is a significant finding. Stx2 was known as more frequent virulence factor among STEC isolates. Our results emphasize the need to pay more attention to checking and controlling the chicken meat and also antibiotic prescription in veterinaries. Results indicated that the *E. coli* virulence genes especially *stx1, stx2, eaeA,* are well distributed in pathogenic *E. coli* strains isolated from poultry meat and the O157 serogroups are the predominant serogroups of EHEC in chicken meat in Hamadan city. Finally, our results indicates to the utility of molecular techniques based PCR for detection and molecular typing of pathogenic *E. coli* strains.

Limitations

One of the most important limitations of this study was the low number of *Escherichia coli* isolated from row meat. More sampling is required for molecular studies. We have also limitations in financial support.

Abbreviations

E. coli; Escherichia coli

STEC: Shiga toxin producing *Escherichia coli*

eae: Escherichia coli attaching and effacing or Intimin

stx1 and stx2; Shiga toxin1 and 2.

hly; hemolysin

GM; gentamicin, CIP; ciprofloxacin, AN; amikacin, A; amoxicillin, MEN; meropenem

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, LS, participated in data analysis. All authors read and approved the final manuscript.

Author details

[1]Student Research Committee, Hamadan University of Medical Sciences, Hamadan, Iran.

[2] Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, P.O box: 6517838678, Hamadan, Iran.

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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 Hamadan University of Medical Sciences, Institutional Review Board (IR.UMSHA.REC.1398.12).

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Figures

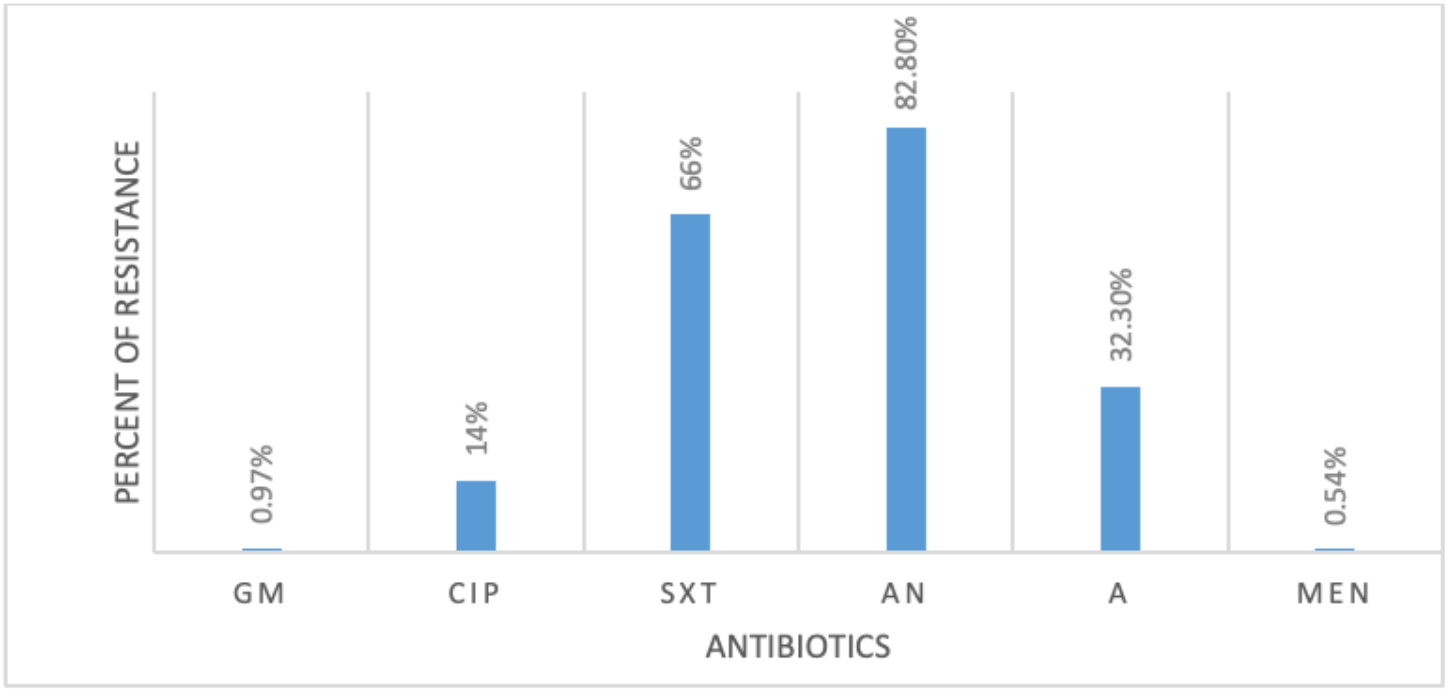

Figure 1

Antimicrobial resistance rates (%) of E. coli strains isolated from row chicken meats

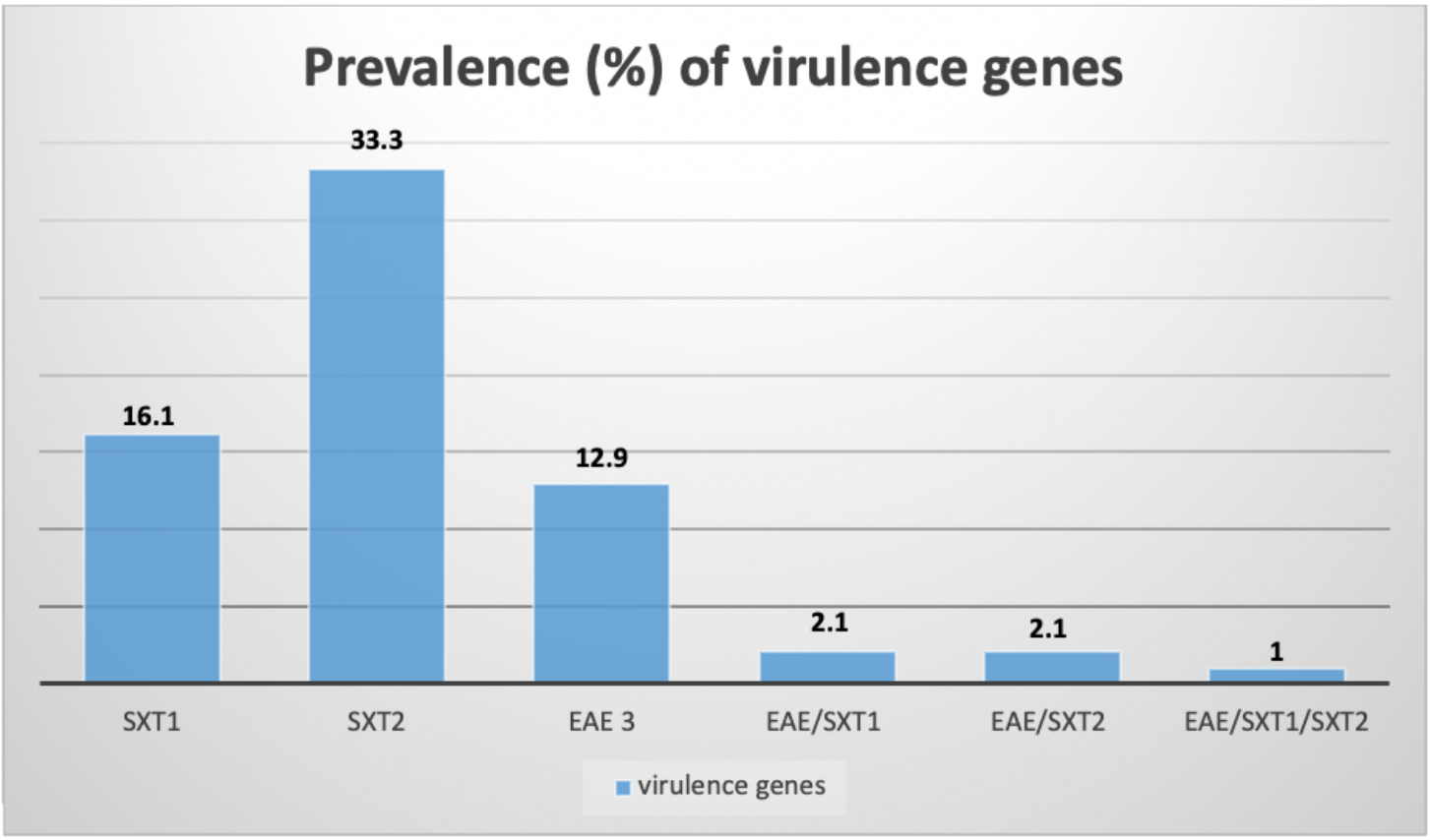

Figure 2

Prevalence of virulence genes among 93 E.coli isolates which isolated from row meat chicken

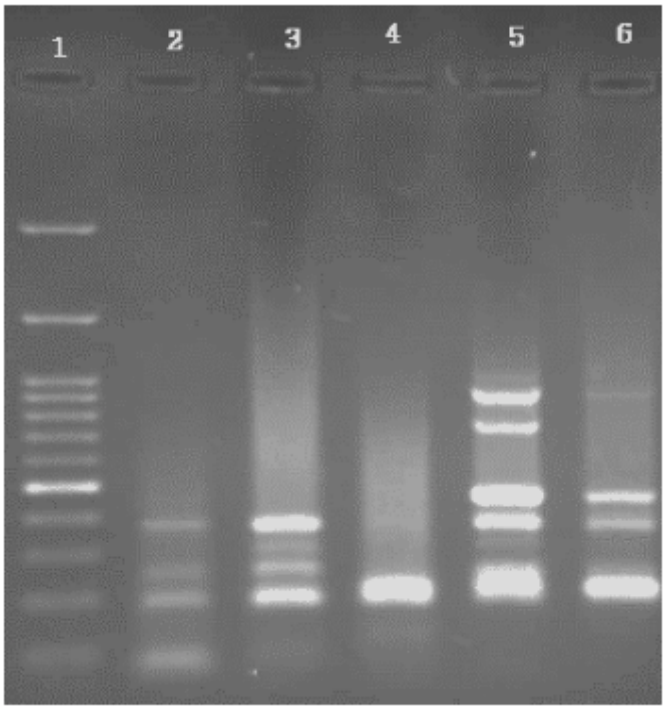

Figure 3

Different ERIC patterns of 5 different EHEC isolates: Lane 1: ladder, lane 2-6: EHEC