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Antibiotic Resistance in Food Poisoning Caused By Escherishia Coli O157:H7 in Hospitalized Patients At 5 Years in Iran

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ABSTRACT

Escherishia coliis bacteria that normally live in the intestines of humans. One particular Escherishia colistrain called 0157:H7 can cause severe diarrhea and kidney damage. The two major aims of this study were to detect this bacteria in stool of diarrheic patients and comparison between phenotypic and genotypic characterization of antibiotic resistantEscherishia coli0157:H7 strainin Iran. 325 diarrheal samples were collected through 2009-2013. Microbiological examinations were done to detect the E. coli O157:H7. PCR was used to identify dfrA1, sul1, citm,tetA, qnrgenes. Antibiotic resistance test was performed. Totally, 57 out of 325 sampleswere found to be contaminated withE. coli. Results showed the lowest resistance was for tetracycline (12.28%) while the highest resistance was for trimethoprim (71.92%). The resistance to sulfametoxazole, ciprofloxacin andampicillin was found in61.40%, 17.54% and47.36% of E. coli O157:H7 strains, respectively. The results of PCR showed 10 isolates contain sul1, 22 isolates containcitm, 6 isolates containtetA, 36 isolates containdfrA1, 9 isolates containqnr genes.Comparison between the phenotypic and genotypic of isolates revealed citm,tetA,dfrA1, qnr and sul1genes covered 38.59%, 10.52%, 63.15%, 15.78% and17.54%of the antibiotic resistances, respectively.

Keywords: Escherishia coli O157:H7 , PCR,diarrhea.

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INTRODUCTION

Escherichia coli (E. coli) is a gram-negative, rod-shaped, flagellated, nonsporulating, and facultative anaerobic bacterium which belongs to Enterobacteriaceae Family. This bacterium classified into several categories based on its virulence factors such asenteropathogenicE. coli (EPEC), enterotoxigenicE. coli (ETEC), enteroinvasiveE. coli (EIEC), enteroaggregativeE. coli (EAEC), enterohemorrhagicE. coli (EHEC or STEC), diffuseadheringE. coli (DAEC).

There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. Domestic animals especially calves are the main sources of bacterium. The E. coliO157:H7 usually transfer to human after consumption of uncooked meat, raw milk and contaminated vegetables and water.Antibiotic resistance has been regarded in many countries [1]. It has been demonstrated that diarrheagenicE. colistrains are categorized into specific groups based on the virulence properties, mechanisms of pathogenicity, clinical syndromes, and distinct O:H serotypes [2]. A number of E. coli strains are recognized as important pathogens of colibacillosis in poultry and some of them can cause severe human diseases such as haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). EnterotoxinogenicE. coli is a common cause of haemolytic uremic syndrome in developing countries. The enteric pathogens are often resistant to multiple antibiotics. People infected by *E. coli* 0157:H7 can develop a range of symptoms. Some infected people may have mild diarrhea or no symptoms at all. Most identified cases develop severe diarrhea and abdominal cramps. Blood is often seen in the stool. Usually little or no fever is present.

However, a large number of outbreaks of enterotoxins have also been associated with consumption of contaminated drinking water or contact with recreational waterGastrointestinal diseases, food poisoning, and even death in some cases make this microorganism an insidious threat to human health and food safety. The E. coli uses as an index for determinding fecal contamination in water and foods [3].

Foods contaminated with antibiotic resistant bacteria could be a major threat to public health. Trough outbreaks in the European Union [4] and several reports of septicemia in human and animals [5, 6] up to recent studies in England [7], all of them have doubled the importance of addressing the issue.Thegenes encoding antibiotic resistance determinants are carried on mobile genetic elements.These genes may be transferred to other bacteria of human clinical significance.The E. coli is a candidate agent for such transfers because of its diversity and also because it survives as common flora in the gastrointestinal tracts of both humans andanimals. They are sensitive to selection pressure exerted by antibiotic usage and carry genetic mobile elements to achieve such transmission [8]. In addition, the lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems increases the risk of foodborne microbes harboring an array of resistance genes. The treatment of food poisoning caused by E. coli O157:H7 often requires antimicrobial therapy. The decision to use antimicrobial therapy depends on the susceptibility of the microorganism and the pharmacokinetics of the drug foridentifying the desired therapeutic concentration.However antibiotic-resistant strains of bacteria cause more severe diseases for longer periods of time than their antibiotic-susceptible counterparts. Several studies showed that antibiotic resistance in E. coli

O157:H7is increasing in these days [9]. Therefore, identification of resistance genes of bacteria seems to be so essential in reduction of treatment costs. Therefore, present study was carried out in order to comparison the phenotypic and genotypic characterization of antibiotic resistant E. coli O157:H7strains isolated from food poisoning in Iran.

MATERIALS AND METHOD

Sample collection From December 2009 to January 2013, a total of 325 diarrheal fecal samples were collected from the hospitalized patients with food poisoning in shahrekord and Isfahan in Iran. The questionnaire was prepared by the team containing information like sex, age, job,etcand was filled by patients.

Isolation of E. coli Macconky agar (McA, Merck, Germany) and Salmonella Shigella agar (Ss, Merck, Germany)and Sorbitol agar (Merck, Germany) were used to detect E.coli. A swab of fecalsample was cultured on McA and Ss and sorbitol agar and incubated for 24 h at 37°C. Complete biochemical identification (Gram staining, oxidase negative, indole positive,Simon's citrate negative and urease negative) was used to confirm the E. coli O157:H7. Bacteriological examinations were done on non lactose fermenting colonies to confirm major causes of diarrhea e.g. Salmonellaeand Shigella(10). The colonies were confirmed for E. coliO157:H7typic were extracted using Genomic DNA in Polymerase Chain Reaction (PCR).

Primers located at the $3^{'}$ conserved segment were used as described by Sandvang and Aarestrup (Table1) [8].

Target	Sequence(5-3)	Size	Tempracher
Sul1-F	TTC GGC ATT CTG AAT CTC AC	822	46
Sul1-R	ATG ATC TAA CCC TCG GTC TC	822	46
CITM-F CITM-R	TGG CCA GAA CTG ACA GGC AAA TTT CTC CTG AAC GTG GCT GGC	462 462	48 48
TetA–F TetA-R	GGT TCA CTC GAA CGA CGT CA CTG TCC GAC AAG TTG CAT GA	577 577	55 55
dfrA1-F dfrA1-R	GGA GTG CCA AAG GTG AAC AGC \GAG GCG AAG TCT TGG GTA AAA AC	367 367	48 48
Qnr-F Qnr-R	GGG TAT GGA TAT TAT TGA TAA AG \CTA ATC CGG CAG CAC TAT TTA	670 670	55 55

Table(1): Primer sequences used in PCR and expected sizes of product

EXPERIMENTAL

DNA was extracted from E. coliO157:H7(Obtained from Department of Microbiology, Faculty ofMed, Tehran University) using Genomic DNA purification kit (Fermentase, Germany) and used as template for standard control in PCR [11].

Total DNA of the isolates were extracted using Genomic DNA purification kit (Fermentas, Germany). The isolated DNA was resuspended in 50 ul of Tris-EDTA (TE) buffer at pH 8. Two microlitre of elute was used as DNA template in PCR assay. PCR reactions were performed in a total volume of 25 µl, including 1.5 mM $Mgcl_2$, 50 mMKcl, 10mMTris-Hcl(PH 9.0), 0.1% Triton X-100, 200µm of each dNTP (Fermentas), 1µm primers 1 Iu of Taq DNA polymerase (Fermentas), and 5µl (40-260 ng/µl) of DNA. Amplification reaction were carried out using a DNA thermo-cycler (Eppendrofmastercycler, Eppendrof-Nethel-HinzGmblt, tlamburg, Germany) as follows:three min at 95◦C, 35 cycles each consisting of 1 min at 94◦C, 90s at ~55◦C (show in Table2) and 1 min at 72◦C followed by a final extension step of 10 min at 72◦C. Amplified samples were analyzed by electrophoresis in agarose gel and stained by ethidium bromide. A molecular weight marker with 100bp increments(100bp DNA ladder, Fermentas) was used as a size standard. In PCR were used 5 primer sets (Cinagen, Iran) to identify virulence genes including dfrA1, sul1, citm,tetAandqnr. PCR was performed as described previously [12] and amplified DNA fragments were resolved by gel electrophoresis using 2 percent agarose and stained with ethidium bromide(8)The presence of genes associated with resistance to ampicilin (Citm), tetracycline (tetA) trimethoprim (dfrA1), quinolones(qnr)and sulfonamides (sul1) were determined by PCR and the set of primers used for each gene.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was carried out using the disk diffusion method according to the recommendations reported by the National Committee for Clinical Laboratory Standards (CLSI). As recommended by the NCCLS, Mueller-Hinton agar batches were used as the culture medium. The antimicrobial agent discs were obtained from CinagenLaboratoy (Tehran, Iran) in Iran. Isolates were tested against commonly used antibiotics such as: ciprofloxacin (CP), sulfamethoxazole (SXT), tetracycline (T), ampicillin (AM) and trimethoprim (TMP). The zone diameters around all disks were interpreted by using the recommendations of the CLSI. E. coliATCC 25922 was used as quality control organisms in antimicrobial susceptibility determination [13, 14].

RESULTS

Out of 325 samples, 57 samples (17.5%) were confirmed as E. coli O157:H7 by biochemical and microbial tests All of these positive E. coli O157:H7 isolates were confirmed using the PCR technique. Results showed that the least resistance was for tetracycline (12.28) and the most resistance was founding for trimethoprim (71.92). The resistance tosulfametoxazole, ciprofloxacin andampicillin was found in 61.40, 17.54 and47.36respectively(Table2).

Antimicrobial Agent	(I)	(S)	(R)
Tetracycline	35.08	52.63	12.28
Ampicillin	17.54	35.08	47.36
Ciprofloxacin	29.82	52.63	17.54
Sulfametoxazole	21.05	17.5	61.40
Trimethoprim	12.28	15.78	71.92

Table (2): Distribution of antibiotic resistance in E. coli strains isolated from patients with diarrhea.

Typ of sample	Number	Isolates Of E. coli O157:H7	Isolates contain Sul1	Isolates contain CITM	Isolates contain TetA	Isolates contain dfrA1	Isolates contain Qnr
Stool Diarrhea	325	57	10sample (17.54%)	22Sample (38.59%)	6sample (10.52%)	36sample (63.15%)	9sample (15.78%)

Table (3): Presences of antibiotic resistance genes in E. coli strains isolated from diarrheic samples in Iran

Results of Comparison between phenotypic and genotypic of the isolates were have showed in Table 3.

DISCUSSION

Different use patterns of antimicrobial agents are expected to have some impact on thedistribution of antimicrobial resistance phenotypes (15) and possibly of resistance determinants. Our data on the distribution of resistance phenotypes in the populations studied here support this hypothesis.For instance, first generation sulfametoxazole are heavily used for the treatment of bacterial infections and particularly of diarrhea (16). This use is clearly reflected in the higher resistance rate for sulfametoxazole observed in human. The antimicrobial resistance may be as a result of inappropriate and wide use of different antibiotics to treat infection. The widespread use of these antibiotics has resulted in an increased prevalence of resistance to these antibiotics by diarrheagenicbacteria, there is raising concern among veterinarian and general practitioners and pediatricians especially in developing countries (17).Our findings showed that the E. coliO157:H7were the major cause of human infections in this area of Iran.Among identified dietary risk factors, foods of bovine origin, particularly undercooked ground beef, have been implicated as a frequently source. These results are in similar with previous report from Iran cited by the World Health Organization (WHO),Bouzari (18) observed thatsulfamethoxazole-trimethoprim, tetracycline were 112 (80.0%), 90 (64.3%) of the diarrheagenic E. coli isolates were resistant to these antibiotics, respectively.

In another investigation in Egypt reported that the occurrence of antibiotic resistance among E. coliO157:H7 isolates from patients with acute diarrhea was 68.2%, 57.2% and 24.2% for ampicillin, trimethoprim-sulfamethoxazole and ampicillin-sulbactam, respectively.

The mechanism of spread of antibiotic resistance from food with animal origin to humans remains controversial. This suggests that silent gene cassettes for antimicrobial agents other than sulfonamides could already be present in these populations and may play a role in the future in the emergence of new resistance phenotypes.

CONCLUTION

Thus, the search for resistance determinants in future studies should not be limited to phenotypically resistant isolates, but should also take susceptible isolates into consideration. However,doctors have a limited choice of antimicrobials for use in human due to antimicrobial resistance issues and human health concerns.Momtaz (13)in the PCR results ofa study in 2012 in Iran showed that *tetA, tetB*were the highest (64.70%) and *aac*(3)-*IV* were the lowest (27.45%) antibiotic resistant genes in *E. coli* positive samples.Van (19)in a study in 2008 inVietnam showed the resistance to tetracycline, ampicillin, ciprofloxacin, sulfametoxazole, trimethoprim and gentamicin was found in 77.8%, 50.5%, 16.2%, 60.6%, 51.5% and 24.2% of E. coli strains.

The results of the present study showed that prescription of sulfametoxazole and trimethoprim due to their high resistances are not reasonable. This study showed that 71.92% and 61.40% of E. coliO157:H7 had resistance to trimethoprim and sulfametoxazole, respectively.Our study indicated the irregularandunauthorizeduse ofantibioticsin medical treatment in Iran. Unfortunately, doctors in their treatment protocols, use antibiotics as a basic one. Therefore, ina very shortperiod of time, antibiotic resistancewill appear.We recommended the PCR technique in order to detection the antibiotic resistance genes of E. coli strains isolated from diarrheic samples. This procedure has clinical and laboratorial importance. Finally, using from disk diffusion method can help to reduce the antibiotic resistance.

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