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RESEARCH ARTICLE

Antibiotic resistance of enterotoxigenic and entroaggrigative *Escherichia coli* isolated from gastroenteritis cases

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ABSTRACT

Background: Toxigenic strains of *Escherichia coli* are common enteric pathogens of human. The aim of this study was to detect virulence genes and antibiotic resistance pattern of the enterotoxigenic and enteroaggrigative *E. coli* isolated from diarhoeal stool samples.

Methods: Totally, 234 diarrhoeal stool samples were collected. Microbiological examinations were done to detect the *E. coli*. PCR was used to identify *Lt, Sta, Stb* and *East1* genes. Antibiotic resistance test was performed using the Disk diffusion method.

Results: Out of 114 isolated *E. coli*, 15(13.5%) harbored *Stb*, 52(45.61%) *East1*, 30 (26.31%) *Lt* gene. The *Sta* gene was not detected in tested samples. The lowest resistance was for gentamicin (0%) while the highest resistance was for trimethoprim (79.8%). Resistance of *E. coli* isolates to chloramphenicol, cephotaxime, sulphametoxazole, ciprofloxacin, ampicillin and tetracycline were 3.5%, 7.01%, 71.05%, 10.5%, 52.63% and 3.5%, respectively.

Conclusions: Toxigenic *E. coli* strains perticipated in diarrhoea in Shahrekord-Iran. The high presences of antibiotic resistance have been shown to trimethoprim, sulphametoxazole and ampicillin.

Keywords: Virulence Genes, Toxigenic *Escherishia coli*, Antibiotic resistance pattern, stool, Iran.

1. INTRODUCTION

Escherichia coli (*E. coli*) is generally considered as a commensally member of the normal intestinal micro flora in humans and animals. *E. coli* strains are categorized into specific groups based on virulence properties, mechanisms of pathogenicity, clinical syndromes and distinct O:H serotypes (1). The main six categories include enteropathogenic *E. co*li (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC or STEC), diffuse adhering *E. coli* (DAEC) (2). Enterotoxinogenic *E. coli* is a common cause of diarrheal diseases in developing countries. Since, the ETEC is a major cause of traveler's diarrhea. Toxins appear to be transmitted primarily through the ingestion of fecal contaminated foods (3). However, a large number of outbreaks of enterotoxins have been associated with consumption of contaminated drinking water or contact with recreational water (4). Recently large outbreak of dysentery complicated by haemolyticuraemic syndrome (HUS) has been observed in north Germany (5). The World Health Organization (WHO) confirmed that this previous outbreak in Germany was related to infection by new and unusual enteroaggregative Shiga toxin/verotoxin-producing *E. coli* O14:H4 strain (5,6). *E. coli* uses as an index for determining fecal contamination in water and foods. There is worldwide concern about the appearance and rise of bacterial resistance to commonly used antibiotics. In this regard

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program for monitoring resistance have been implemented in many countries (7,8, 9).

Foods contaminated with antibiotic resistant bacteria could be a major threat to public health. There is a distinct possibility that genes encoding antibiotic resistance determinants carried on mobile genetic elements may be transferred to other bacteria of human clinical significance.

E. coli is a candidate vehicle for such transfers because of its diversity and survives as common flora in the gastrointestinal tracts of both humans and animals (10). In addition, the lack of stringent controls on antimicrobial usage in human health and particularly in animal production systems increases the risk of foodborne pathogens harboring an array of resistance genes.

This study was conducted to identify the enterotoxigenic and enteroaggrigative *E. coli* and determine the profile of antimicrobial resistance of the isolated strains from patients with diarrhoea in south west of Iran (Shahrekord province, Iran).

2. MATERIAL AND METHODS

2.1. *Sample collection and isolation of E. coli*

A total of 234 fecal samples from the patients affected with diarrhoea were collected in Hajar hospital of Shahrekord, Iran, during December 2011 to January 2012. Macconky agar (McA) and salmonella shigella agar (SSA), (Merck, Germany), were used to detect *E. coli*. A swab of fecal sample was cultured on McA and SS agar and incubated for 24 h at 37 ^{0}C. Complete biochemical identification (Gram negative, oxidase negative, indole positive, Simon's citrate negative and urease negative) was used to confirm the *E. coli*. Bacteriological examinations were done on non lactose fermenting colonies to confirm major causes of diarrhea e.g *Salmonellae* and *Shigella*. The colonies were confirmed using Polymerase Chain Reaction (PCR) based on the detection of 16S rRNA gene region of *E. coli* described by Sabat et al. (Sabat et al., 2000).

Sabat, G., P. Rose, W. J. Hickey, and J. M. Harkin. 2000. Selective and sensitive method for PCR amplification of *Escherichia coli* 16S rRNA genes in soil. Appl. Environ. Microbiol. 66:844-849.

2.2. Detection of sta, stb, Lt and East1 genes

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 micro liter 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 mM Kcl, 10mM Tris-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 (Eppendrof mastercycler, Eppendrof-Nethel-Hinz Gmblt, 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 Table1) 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 4 primer sets (Cinagen, Iran) to identify virulence genes including *sta, stb, Lt and East*1. PCR was performed as described previously (11) and amplified DNA fragments were resolved by gel electrophoresis using 2 percent agarose and stained with ethidium bromide.

E. coli strain O149:K91 (Reference Laboratory for *E. coli*, Faculte de medicine veterinaire, Universite de Montreal) using Genomic DNA purification kit (Fermentase, Germany) and used as template for standard control in PCR.

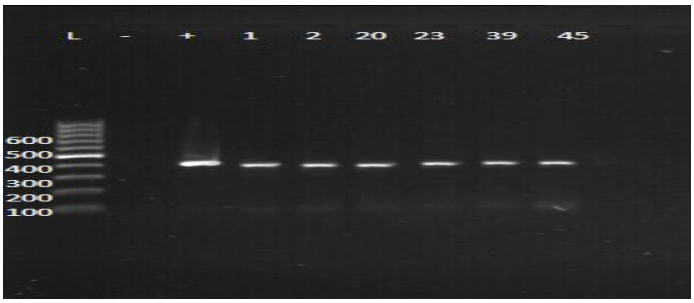
2.3. Antimicrobial susceptibility testing

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 Cinagen Laboratoy (Tehran, Iran) in Iran. Isolates were tested against commonly used antibiotics such as: ciprofloxacin (CP), sulfamethoxazole (SXT), tetracycline (T), gentamycine (GM), cephotaxime (CN), chloramphenicol (C), ampicillin (AM) and trimethoprim (TMP). The zone diameters around all disks were interpreted by using the recommendations of the CLSI. *E. coli* ATCC 25922 was used as quality control organisms in antimicrobial susceptibility determination.

3. RESULTS:

Out of 234 samples, 114 samples (48.1 %) were confirmed as *E. coli* by biochemical and microbial tests. All of these positive *E. coli* isolates were confirmed using the PCR technique. Of the 114 *E. coli* isolates, 15 (13.5%) were detected as *STb* carrying *E. coli* (Figure 1).

Fig 1: PCR detection of *Stb* gene of *E. coli* M (DNA marker), PC (positive control) and NC (negative control).



In total 52 (45.61%) samples carried *East1* gene (Figure 2) and 30 (26.31%) samples carried *LT* (Figure 3). None of the

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isolates had *STa* gene (Figure 4). Also the results showed that 1 (0.87%) isolates contain both *LT* and *EAST1*, 2 (1.75%) isolates contain *STb* and *LT* and 3 (2.63%) isolates contain *STb* and *EAST1* genes (Table 2).

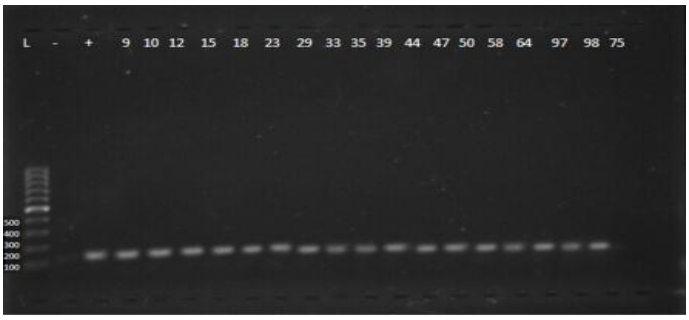


Fig 2: PCR detection of *East1*. M (DNA marker), NC (negative control), PC (positive control).

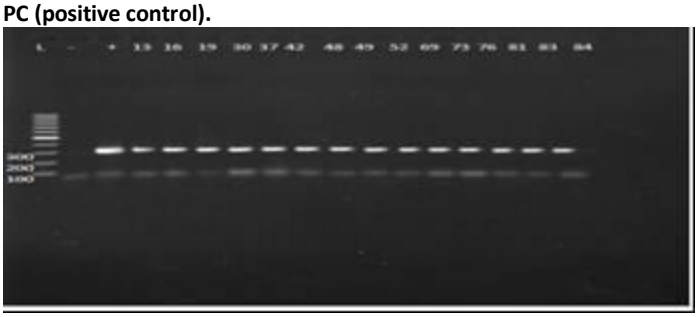
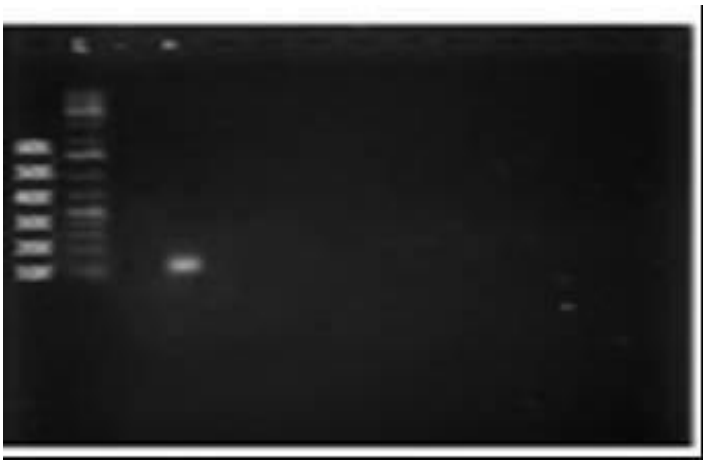


Fig 3: PCR detection of *LT* gene. M (DNA marker), NC (negative control), PC (positive control).



4: PCR detection of *Sta* gene. M (DNA marker), NC (negative control), PC (positive control

The results of antibiotic resistant tests revealed that the lowest resistance was for gentamicin (0%) and the highest resistance was for trimethoprim (79.8%). The antibiotics resistance for chloramphenicol, cephotaxime, sulfametoxazole, ciprofloxacin, ampicillin and tetracycline were 3.5%, 7.01%,71.05%, 10.5%, 52.63% and 3.5%, respectively. The incidence of multidrug resistance of isolated *E. coli* presented in table 3.

Target	Size (bp)	Annealing Temperature	Primer Sequence
East1	**125**	**55**	**TCGGATGCCATCAACACAGT**
East1-F			**GTCGCGAGTGACGGCTTTGTAG**
East1-R			
Sta	**163**	**60**	**TCCCCTCTTTTAGTCAGTCAACTG**
Sta-F			**GCACAGGCAGGATTACAACAAAGT**
Sta-R			
Stb	**368**	**60**	**GCAATAAGGTTGAGGTGAT**
Stb-F			**GCCTGCAGTGAGAAATGGAC**
Stb-R			

Table1: Primers sequences used in PCR and expected sizes of products

Isolates	*STb*	*LT*	*EAST1*	*LT, EAST1*	*STb, LT*	*STb, EAST1*
114	15(13.15)	30(26.31)	52(45.61)	1(0.87)	2(1.75)	3(2.63)

Table 2: Incidence of virulence genes of *E. coli* isolated from diarrhoeal cases.

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4. DISCUSSION

Toxigenic *E. coli* is the most common bacterial etiologic agent of diarrhoea in human and animals in developing Countries (12). Although treatment of enteric *E. coli* infection include the use of antimicrobial agents but increasing resistance to first-line of antibiotics represents a potential threat to human and animal health (13,14,15). The antimicrobial resistance may be as a result of inappropriate and wide use of different antibiotics to treat infection. Resistance to currently used antimicrobial agents among enteric pathogens has increased dramatically worldwide during the past decade (11, 16, 17). In developing countries, trimethoprim-sulfamethoxazole, ampicillin and tetracycline are widely used antibiotics in human to treat diarrhoea because of their low cost and availability (18). The widespread use of these antibiotics has resulted in an increased prevalence of resistance to these antibiotics by diarrheagenic bacteria; there is raising concern among veterinarian and general practitioners and pediatricians especially in developing countries (19). This study revealed 85.1% of the *E. coli* strains isolated from diarrhoeal cases contained *EAST1, STb* and *LT* genes.

Resistance	NO of isolates	Percentage
Single	20	17.5
Double	45	39.5
Triple	44	38.4
Quadruple	5	4.4

Table 3: Incidence of multidrug resistance of *E. coli* isolated from diarrhoeal cases.

It shows that the toxigenic *E. coli* plays an important role in enteritis in this area. Also the resistance to antibiotic tested showed the high resistance to trimetoprim (79.08%), sulfamethoxazole (71.05%) and ampicillin (52.63%). These results are in similar with previous report from Iran cited by the World Health Organization (WHO), sulfamethoxazole-trimethoprim, tetracycline and chloramphenicol were 112 (80.0%), 90 (64.3%) and 78 (55.7%) of the diarrheagenic *E. coli* isolates were resistant to these antibiotics, respectively (12).

Reports from the other countries showed the same results. Studies in Vietnam revealed that 86.4%, 77.2% and 19.1% of *E. coli* isolates were resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole, respectively (18). Another investigation in Egypt showed that the occurrence of antibiotic resistance among *E. coli* isolates from patients with acute diarrhea was 68.2%, 57.2% and 24.2% for ampicillin, trimethoprim-sulfamethoxazole and ampicillin-sulbactam, respectively (20).

This current study showed the high multidrug resistance among toxigenic *E. coli* isolated from enteritis cases to trimetoprim, sulfametoxazol and ampicillin. Several studies have determined that the multi-drug resistance is common among *E. coli* isolates, especially to ampicillin, trimethoprim-sulfamethoxazole and tetracycline (21,22). In contrast, Chattopadhyay et al, (2001) reported the antibiotic resistance pattern of STEC strains isolated from animal, human and food products to tetracycline, cephalexin, cloxacillin, erythromycin and lincomycin. The changing patterns of resistance to common antimicrobial agents in Iran indicates that designing a surveillance system for antimicrobial resistance and the introduction of integrated guidelines for the appropriate use of antibiotics are urgently needed.

The results of this study suggest that antimicrobial resistance is widespread among potentially diarrhoeagenic *E. coli* strains. Our results showed the close relation between the presence of virulence genes and antibiotic resistance in resistant strains. The results of our study are supported by previous studies indicating that *E. coli* virulence factors could be the reason for resistance to different antibiotics (23,24). It can be concluded that emergence and dissemination of antimicrobial resistance in *E. coli* strains containing virulence factors may complicate treatment of certain enteric or urinary tract infections in humans and animals.

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6. REFERENCES:

1. Meng J, Doyle MP. Enterohemorrhagic Escherichia coli In:Doyle, M.P., Beuchat, M.P., Montville, T.J. (Eds.), Fundamentalsand Frontiers. ASM Press, Washington D. C. Food Microbio. 2001; 2: 193–213.
2. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. Clin Microbiol Rev. 1998; 11: 142–201.
3. Frankel G, Phillips AD. Generation of E.coli derivatives with differing biological activities using site-directed mutagenesis of the intimin C-terminus domain. Mol Microbiol. 1998; 29: 559-570.
4. Keene WE, McAnulty JM. A sweeming associated outbreak of hemorrhagic colitis caused by E.coli O157:H7 and Shigellasonnei. Engl J Med. 1994; 331: 579-584.
5. Loos S, Ahlenstiel T. An Outbreak of Shiga-Toxin Producing E. coli O104:H4 Hemolytic Uremic Syndrome (STEC-HUS) in Germany: Presentation and Short-term Outcome in Children. Clin Infect Dis. 2012; 15: 15-38.

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6. Simon K, Janocha J. Epidemic of EHEC (Escherichia coli O104:H4) in Europe in 2011. clinthera problems. Przegl Epidemiol. 2012; 66(1):73-7.
7. Badri S, Fassouane A. Relationship between susceptibility to antimicrobials and virulence factors in Escherichia coli isolated from food in Morocco. Int J Food Saf. 2009; 11: 98-101.
8. Karmali MA. Infection by verocytoxin-producing E.coli. Clin Microbiol Rev. 1989; 2(1): 15-38.
9. Munisa M, Jofre J. Abundance in sewage of bacteriophages that infect E.coli O157: H7 and that carry the shiga toxin 2 gene. Appl Environ Microbiol. 1998; 64: 2443-2448.
10. Van den Bogaard AE, Stobberingh EE. Epidemiology of resistance to antibiotics
11. between animals and humans. Int J Antimicro Agents. 2000; 14: 327–335.
12. Salmanzadeh-Ahrabi S, Jafari F. Serotype distribution and antimicrobial resistance rates of Shigell isolates in Tehran, Iran. Microbiol Bull. 2007; 41:453-7.
13. Bouzari S, Jafari A. Distribution of genes encoding toxins and antibiotic resistance patterns in diarrheagenic Escherichiacoli isolates in Tehran. East Med Health. 2007; 13, 287-293.
14. Diarra MS, Silversides FG. Impact of feed supplementation with antimicrobial agents on growth performanceof broiler chickens, Clostridium perfringens and Enterococcuscounts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in Escherichia coli isolates. Appl Env Microbiol. 2007; 73: 6566–6576.
15. Johnson TJ, Siek KE. DNA sequence and comparative genomics of pAPEC-O2-R, an avian pathogenic Escherichia coli transmissible R plasmid. Antimicro Chemother. 2005; 49: 4681– 4688.
16. Russo TA, Johnson JR. Medical and economic impact of extraintestinal infections due to Escherichia coli: focus on an increasingly important endemic problem. Micro Infect. 2003; 5:449–456.
17. Temu MM, Kaatano GM. Antimicrobial susceptibility of S. flexneriandS. dysenteriaeisolated from stool specimens of patients with bloody diarrhoea in Mwanza, Tanzania. Tanzanian Heal Res Bull. 2007; 9: 186-9.
18. Woodward DL, Rodgers FG. Surveillance of antimicrobial resistance in Salmonella, Shigella and Vibrio choleraein Latin America and the Caribbean: A collaborative project. Can J of Infects Dis. 2000; 11: 181-184.
19. Nguyen TV, Le PV. Antibiotic resistance in diarrheagenic Escherchia coli and Shigella strains isolated from children in Hanoi, Vietnam. Antimicro Agents Chemother. 2005; 49: 816-9.
20. Vila J, Vargas M. Antimicrobial resistance of diarrheagenic Escherichia coli isolated from children under the age of 5 years from Ifakara, Tanzania. Antimicro Agents Chemother. 1999; 31: 3022-4.
21. Putnam SD, Riddle MS. Antimicrobial susceptibility trends among Escherichia coli and Shigella spp. isolated from rural Egyptian paediatric populations with diarrhea between 1995 and 2000. Clin Microbiol. 2004; 10: 804-10.
22. Aslani MM, Salmanzadeh-Ahrabi S. Molecular detection and antimicrobial resistance of diarrheagenic Escherichia coli strains from diarrheal cases. J Biol Sci. 2008; 20: 388-92.
23. Lolekha S, Vibulbandhitkit S. Response to antimicrobial therapy for shigellosis and colibacillosis in Thailand. Rev Infect Dis.1991; 13: S342-6.
24. Badri S, Fassouane A. Relationship between susceptibility to antimicrobials and virulence factors in Escherichia coli isolated from food in Morocco. Int J FoodSaf. 2009; 11: 98-101.
25. Orden J, Ruiz-Santa-Quiteria JA. In vitro susceptibility of Escherichia coli strains isolated from diarrhoeic dairy calves to 15 antimicrobial agents. J of Vet Med B (Infect Dis Vet Public Health). 2000; 47: 329-335.

Conflict of Interest: None Declared

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