Research in Pharmaceutical Sciences, April 2007; 2: 29-33
Received: Oct 2006
Accepted: March 2007

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Antibiotic resistance pattern in *Shiga* toxin-producing *Escherichia coli* isolated from diarrheal patients in Al-zahra Hospital, Isfahan, Iran.

H. Fazeli[1,*] and R. Salehi[2]

[1]*Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, I.R.Iran.*
[2]*Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, I.R.Iran.*

Abstract

Shiga toxin-producing *Escherichia coli* (STEC) can cause a broad spectrum of human illness. The progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. The aim of current study was to determine bacterial resistance pattern of a group of diarrheagenic *E. coli* to some important antibiotics. The research method was descriptive and observational. Isolates of STEC were assessed for susceptibility based on minimum inhibitory concentration method (MIC). MICs were determined by the broth dilution method following the recommendations of the National Committee for Clinical Laboratory, Standards (NCCLS).Of the 29 STEC isolates, 65.5, 72.4, 58.6, 27.6, 13.8, 20.7, 6.9 percentage were resistant to amoxicillin, trimethoprim-sulfamethoxazole, tetracycline, cefazolin, ceftriaxon, nalidixic acid and ciprofloxacin respectively and all were sensitive to ofloxacin. More than 66% of STEC strains were resistant to the 3 commonly used antibiotics with high MIC_{90}s. According to our study, amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole are not recommended for the treatment of diarrhea in this population. Therefore, regional information regarding antibiotic resistance should be used in clinical management, and treatment guidelines should be updated

Keywords: Shiga; Escherichia coli; Minimum inhibitory concentration; Antibiotic resistance

INTRODUCTION

Antibiotics have revolutionized the treatment of common bacterial infections and play a crucial role in reducing mortality. Antimicrobial therapy should be used in severe cases of diarrheal disease to reduce the duration of illness and may also use to prevent traveler's diarrhea (1). However, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. In addition, the overuse and misuse of antibiotics in the treatment of diarrhea could lead to an increase in antibiotic resistance. Many patients with symptoms of illnesses diarrhea, may have been empirically treated with antibiotics without advice from medical staffs (2-4).

According to the epidemiological studies in our countries, *E. coli* has been the most commonly isolated in clinical samples from patients with diarrhea and shows a high prevalence of resistance (85-100%) to antibiotics such as penicillin, erythromycin, tetracycline (5,6). Rezaie et al. (6) reported that from 161 diarrhea patients, *E. coli* was isolated in 63 (39%), among which 83 pathogenic specimens were detected and concluded *E. coli* seems to be a common pathogen in Varamin,

*Corresponding author: Dr. H. Fazeli
Tel. 0098 311 7922439, Fax. 0098 311 6680011
Email: h_fazeli@med.mui.ac.ir

however, due to the lack of facilities, it is not routinely checked in laboratories. In another study conducted by Zali and his co-workers, it was found that *E. coli* species have been the most frequently isolated pathogen in Iran, followed by *Salmonella*, *Shigella*, and *Campylobacter*; while in developed countries, *Campylobacter* has been more prevalent (5). One group of *E. coli* that causes diarrhea is Shiga toxin-producing *E. coli* (STEC). STEC has emerged as food-born pathogen that can cause severe and potentially fatal human illnesses. They are the major cause of gastroenteritis that may be complicated by hemorrhagic colitis or the hemolytic uremic syndrome, which is the main cause of acute renal failure in children (7,8).

Information about antimicrobial resistance among STEC and other pathogenic *E. coli* in Iranian patient are scarce (5,7). In this study, the susceptibility of 29 STEC strains to different antibiotics are evaluated.

MATERIALS AND METHODS

Bacterial strains

Twenty-nine STEC bacteria were isolated with microbiological methods and confirmed by polymerase chain reaction. Isolated bacteria cultured in 15% glycerol, 0.6% peptone, water and stored at -20 ^{o}C.

Antimicrobial agents

The following antibiotics were used for susceptibility testing: amoxicillin (Sigma), tetracycline (Merck), trimethoprim-sulfamethoxazole (Merck), ofloxacin (Merck), cefazolin (Sigma), ceftriaxon (Sigma), nalidixic acid (Merck), and ciprofloxacin (Sigma). Solvents and diluents for preparation of stock solutions of antimicrobial agents are shown in Table 1.

Table 1. Solvents and diluents for preparation of stock solutions of antimicrobial agents

Antimicrobial Agent	Solvent	Diluent
Amoxicillin	Phosphate buffer, pH 6.0, 0.1 M	Phosphate buffer, pH 6.0, 0.1 M
Tetracycline	Phosphate buffer, pH 6.0, 0.1M	Water
Trimethoprim	0.05 N HCl	Water (require heating)
Sulfamethoxazol	2.5 M NaOH	Water
Cefazolin	Phosphate buffer, pH 6.0, 0.1 M	Water
Ceftriaxon	Phosphate buffer, pH 6.0, 0.1 M	Water
Nalidixic acid	1 M NaOH	Water
Ciprofloxacin	1 M NaOH	Water
Ofloxacin	1 M NaOH	Water

Susceptibility testing

MICs were determined by broth dilution method by following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS). Serial concentrations of the antimicrobial agents were prepared and placed in tubes of Mueller-Hinton broth medium. A standard inoculum of each isolate of STEC (1×10^6 cfu/ml) was added to 1 ml of each concentration of antimicrobial agent and to a tube of the growth medium without antimicrobial agent, which served as growth control. An uninoculated tube of medium was incubated to serve as a negative growth control. After overnight incubation at 37 ^{o}C, the tubes were examined for turbidity, indicating growth of the STEC. The lowest concentration of the agent that inhibits growth of the *E. coli*, as detected by lack of visual turbidity, was designated the minimum inhibitory concentration (MIC). For calculation of MIC_{50} and MIC_{90}, after the MIC had been determined, a known quantity (0.1 ml) of inoculum from each tube containing broth was subcultured to solid agar plates. The antimicrobial agent that was carried with inoculum was removed by diffusion into agar and the effect was negated by spreading the inoculum over a large area.

The number of colonies that grow on subculture after overnight incubation was then counted and compared to the number

of cfu/ml in the original inoculum. MIC at which 90% and 50% of the isolates tested were inhibited was referred to as MIC_{90} and MIC_{50}, respectively. Susceptibility interpretations were made according to NCCLS. Organisms with MICs at or below the breakpoints (the concentration that can be achieved in the serum with optimal therapy) were considered susceptible.

Analysis of data

Data from antibiotic susceptibility testing were analyzed using SPSS 13 software program. The data were analyzed using Kruskal- Wallis H test for multiple comparisons. P Value of <0.05 was considered significant.

RESULTS

Antibiotic susceptibility testing data are shown in Table 2. Of the 29 STEC isolates, 65.5, 72.4, 58.6, 27.6, 13.8, 20.7, and 6.9 percentage were resistant to amoxicillin, trimethoprim-sulfamethoxazole, tetracycline, cefazolin, ceftriaxon, nalidixic acid and ciprofloxacin, respectively and all were sensitive to ofloxacin (Table 2). The traditional antibiotics, including amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole, showed low activity against STEC. MIC_{90} for amoxicillin, tetracycline and trimethoprim-sulfamethoxazole was 1024, 768, 64 mg/liter, respectively (Table 3).

As indicated in Table 3, cefazolin, ceftriaxon, and nalidixic acid showed moderate activity (MIC_{90} was 512, 64, and 384 mg/liter, respectively). However, MIC_{90}s of cefazolin and nalidixic acid were rather high. They were still more active than the traditional antibiotics but less active than ceftriaxon with a MIC_{90} of 64 mg/liter (Table 3).

The differences in the distributions of resistance and MICs were observed for individual antibiotics. The multiple comparison showed a significant difference in resistance to tetracycline and ciprofloxacin (P = 0.004 and 0.021, respectively). For trimethoprim-sulfamethoxazole, the P value was 0.062. Multi antibiotic resistance (resistance to at least two antibiotics) was detected in 95 % of STEC strains. The most prevalent multi resistance antibiotics were amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole (65.5%, 58.6% and 72.4%, respectively). There was significant difference in antibiotic resistance to nalidixic acid and ciprofloxacin (20.7% and 6.9%, respectively).

Table 2. Antibiotic resistance of 29 *Shiga* toxin-producing *E. coli* isolated from diarrheal patients.

Antimicrobial Agent	No. of resistance	% Resistance
Amoxicillin	19	65.5
Tetracycline	17	58.6
Trimethoprim-sulfamethoxazole	21	72.4
Cefazolin	8	27.6
Ceftriaxon	4	13.8
Nalidixic acid	6	20.7
Ciprofloxacin	2	6.9
Ofloxacin	0	0

Table 3. Minimum Inhibitory Concentration (MIC) including MIC range, MIC_{50} and 90% of 29 *Shiga* toxin-producing *E. coli* isolated from diarrheal patients.

Antimicrobial agents	MIC range	MIC 50%[1]	MIC 90%[2]
Amoxicillin	4-2048	768	1024
Tetracyclin	2-1024	256	768
Trimetoprim-sulfamethoxazole	0.064-128	8	64
Cefazolin	0.125-1024	6	512
Ceftriaxon	0.064-384	4	64
Nalidixic acid	4-768	4	384
Ciprofloxacin	0.016-64	0.032	2
Ofloxacin	0.008-24	0.016	0.5

[1]MIC 50% means MIC at which 50% of the isolates tested are inhibited.

[2]MIC 90% means MIC at which 90% of the isolates tested are inhibited.

DISCUSSION

In the present study, STEC strains were

resistant to the 3 commonly used antibiotics amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole. Some previous studies have shown high prevalence of resistance to these antibiotics in enteric pathogens, especially STEC (12,13). Pour Shafie (19) reported higher percentage of resistance to trimethoprim-sulfamethoxazole and tetracycline in *E. coli* compared to what we have determined. However the resistance against amoxicillin was less than what our data shows (19). Our findings are comparable with the study of Borjian (20) which the resistance against these antibiotics may be due to the higher rate of prescription in treatment of diarrhea because of their low cost and easy application.

According to our findings, amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole are not recommended for the treatment of diarrhea in this population. Therefore, local information about antibiotic resistance should be used in clinical management, and treatment guidelines should be updated (14).

Although the cephalosporins (cefazolin and ceftriaxon) are not indicated for the treatment of diarrhea, we have tested the susceptibilities of STEC strains to these antibiotics, since they could be empirically or incidentally used. In Orrett (21) study in India, an *E. coli* and other gram negatives bacteria the greatest efficacy were observed for imipenem, gentamycin, ciprofloxacin, and the cephalosporins ceftazidime and ceftriaxon. In our study, the results showed that cefazolin and ceftriaxone indicated moderate activity against STEC strains. All STEC strains were susceptible to ofloxacin. This fluoroquinolone (ciprofloxacin, ofloxacin) and quinolone (nalidixic acid) antibiotics are now commonly used to treat infections, including diarrhea. They have also been recommended for prophylaxis and treatment of traveler's diarrhea. Compared to other studies (15,16), our results showed a moderate prevalence of resistance to nalidixic acid and ciprofloxacin in STEC. We have isolated one ETEC strain resistant to both nalidixic acid and ciprofloxacin. Differences between MIC_{50} and MIC_{90} for nalidixic acid and cefazolin found to be much higher than those of others. Therefore, to obtain the best antimicrobial activity of nalidixic acid and cefazolin high concentration of drug must be prescribed to prevent treatment failure.

The multi resistance to amoxicillin, tetracycline, and trimethoprim-sulfamethoxazole were most prevalent. Multi resistance has been also reported in previous studies (12,17,16). Moreover, these *E. coli* had other multi resistance patterns with different prevalences.

We found that 65.5% STEC strains were resistant to amoxicillin, tetracycline, trimethoprim-sulfamethoxazole, and 13.8% were resistant to either nalidixic acid or ciprofloxacin. That means the patients infected with these *E. coli* strains may risk a treatment failure. It is also indicated that the multi resistance of different categories of STEC strains is emerging in our country where these antibiotics (both early and new) have been widely used.

REFERENCES

1. Werber D, Behnke SC, Fruth A, Merte R, Menzler S, Glaser S, et al. Shiga toxin-producing Escherichia coli infection in Germany, different risk factors for different age groups. Am J Epidemiol. 2007;165:425-434.
2. Goldsmid JM, Leggat PA. The returned traveller with diarrhea. Aust Fam Physician. 2007;36:322-327.
3. Dray X, Marteau P. Acute diarrhoea in the adult with treatment. Rev Prat. 2006;56:1811-1816.
4. Van Duong D, Binns CW, Van Le T. Availability of antibiotics as over-the-counter drugs in pharmacies: a threat to public health in Vietnam. Trop Med Int Health.1997;2:1133-1139.

5. Zali MR, Moez AK, Parcham AK, Nikholgh B. Etiologies of acute diarrheal diseases in Iran. J Res Med Sci. 2002;4:356-346.
6. Rezaie Homami M, Salmanzadeh Ahrabi S, Moez Ardalan K. Epidemiology of bacterial-induced acute diarrhea in Varamin. Pejouhandeh Q Res J. 2003;7:474-467.
7. Javadzadeh M, Dabiri S, Zangiabadi M. Role of Shigella, enteroinvasive Escherichia coli (EIEC) and *Entameba Histolytica* in causing dysentery in children and antibiotic sensitivity testing. J Res Med Sci. 2003;39:35-29.
8. Tavakoli A, Pishva E, Fazeli H, Salehi R, Safaei H. Comparison of culture and PCR method in recognition of Escherichia coli O157:H7 in diarrheal patients referred to Al-Zahra hospital in Isfahan. J Isfahan Med School. 2003;21:32-35.
9. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard, NCCLS document M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa; 2000.
10. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; twelfth informational supplement, NCCLS document M100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa; 2002.
11. Nataro JP, Steiner T, Guerrant RL Enteroaggregative Escherichia coli. Emerg Infect Dis. 1998;4:251-261.
12. Hoge CW, Gambel JM, Srijan A, Pitarangsi C, Echeverria P. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. Clin Infect Dis. 1998;26:341-345.
13. Prats G, Mirelis B, Liovet T, Munozc C, Miro E, Navarro F. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. Antimicrob Agents Chemother. 2000;44:1140-1145.
14. Williams R, Ryan MJ. Surveillance of antimicrobial resistance an international perspective. Br Med J. 1998;317:651-660.
15. Gomi, H, Jiang ZD, Adachi JA, Ashley D, Lowe B, Vevenkar MP, et al. In vitro antimicrobial susceptibility testing of bacterial enteropathogens causing traveler's diarrhea in four geographic regions. Antimicrob Agents Chemother. 2001;45:212-216.
16. Odahara Y, Muratani T, Shiozaki K, Kobayashi T, Kawasaki K, Wade A, et al. Antimicrobial susceptibility and mechanism of resistance to antibiotics for Escherichia coli O157 and verotoxin-producing E. coli isolated in northern Kyushu and Yamaguchi area : J Uoeh. 2006; 28:193-201.
17. Lima AA, Lima NL, Pinho MC, Barros EA, Teixeira MJ, Martins MC, et al. High frequency of strains multiply resistant to amoxcillin, trimethoprim-sulfamethoxazole, isolated from patients with shigellosis in northeastern Brazil during the period 1988 to 1993. Antimicrob Agents Chemother. 1995;39:256-259.
18. Sumitaka M, Kyouko K, Hideno T. Outbreak of enterohemorrhagic Escherichia coli O157 attributed to a grilled-meat restaurant. Jpn J Infect Dis. 2006;59:407-408.
19. Pour Shafie MR, Seifi M, Reihani F, Shafiei E, Sedaghat M. Susceptibility pattern of over 1000 pathogens examined at Pasteur Institute. J Shahid Sadooghi Univ Med Sci.1998;4:30-39.
20. Borjian S. Antibiotic susceptibility evaluation of Shigella spp. and entero pathogenic Escherichia Coli associated with children diarrhea. J Zanjan Univ Med Sci Health Services. 1999;27:48-55.
21. Orrett FA, Changoor E. Bacteremia in children at a regional hospital in Trinidad. Int J Infect Dis. 2006;11:145-51.