ORIGINAL ARTICLE

Widespread antibiotic resistance of diarrheagenic *Escherichia coli* and *Shigella* species

Azam Fatahi Sadeghabadi, Ali Ajami, Reza Fadaei, Masoud Zandieh, Elham Heidari, Mahmoud Sadeghi, Behrooz Ataei[1], Shervin Ghaffari Hoseini[2]

Isfahan Provincial Health Center, [1]Nosocomial Infection Research Center, [2]Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Antibiotic resistance of enteric pathogens particularly *Shigella* species, is a critical world-wide problem and monitoring their resistant pattern is essential, because the choice of antibiotics is absolutely dependent on regional antibiotic susceptibility patterns. During summer 2013, an unusual increase in number of diarrheal diseases was noticed in Isfahan, a central province of Iran. Therefore, the antibiotic resistance of diarrheagenic *Escherichia coli* and *Shigella* species isolated were evaluated. **Materials and Methods:** According to the guideline on National Surveillance System for Foodborn Diseases, random samples from patients with acute diarrhea were examined in local laboratories of health centers and samples suspicious of *Shigella* spp. were further assessed in referral laboratory. Isolated pathogens were identified by standard biochemical and serologic tests and antibiotic susceptibility testing was carried out by disc diffusion method. **Results:** A total of 1086 specimens were obtained and 58 samples suspicious of *Shigella* were specifically evaluated. The most prevalent isolated pathogen was *Shigella sonnei* (26/58) followed by *E. coli* (25/58) and *Shigella flexneri* (3/58). A large number of isolated bacteria were resistant to co-trimoxazole (*Shigella* spp: 100%, *E. coli*: 80%), azithromycin (*Shigella* spp: 70.4%, *E. coli*: 44.0%), ceftriaxone (*Shigella* spp: 88.9%, *E. coli*: 56.0%) and cefixime (*Shigella* spp: 85.2%, *E. coli*: 68.0%). About88.3% of *S. sonnei* isolates, one *S. flexneri* isolate, and 56% of *E. coli* strains were resistant to at least three antibiotic classes (multidrug resistant). **Conclusion:** Due to high levels of resistance to recommended and commonly used antibiotics for diarrhea, continuous monitoring of antibiotic resistance seems essential for determining best options of empirical therapy.

Key words: Antibiotic resistance, diarrhea, *Escherichia coli*, Iran, *Shigella*

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INTRODUCTION

Diarrheal diseases with an annual report of approximately 4.6 billion cases, are a common cause of mortality and morbidity world-wide and represent the second leading cause of death in children under 5 years of age.[1] Bacterial dysentery due to *Shigella* species is supposed to be the most complicated form of diarrhea and a large number of associated deaths are reported annually.[2] The burden of diarrheal diseases are far more in developing countries: In a multi canter Asian study, annual incidence of shigellosis was 13.2/1000 children under age 5 year and 2.1/1000 in all ages, which was approximately 100-fold higher than developed countries.[3] Furthermore, antimicrobial resistance of enteric bacteria is rapidly increasing in the developing world.[4] In the past drug resistance was mostly seen in nosocomial infections because of widespread use of antibiotics in hospitals, however recently multidrug resistant bacteria are commonly encountered in community acquired infections, particularly gastrointestinal (GI) infections.[4] Resistant enteric bacteria first emerged in Asia, Africa, and South America, but rapidly spread to developed countries.[5] Extensive resistance of *Shigella* to ampicillin, co-trimoxazole, and nalidixic acid has made these drug no longer useful for empirical treatment of dysentery.[6] Furthermore, resistance to ceftriaxone, azithromycin and ciprofloxacin have been frequently reported in Asia[7] and to a lesser extend in United States.[8] Resistance to low-cost available drugs is a prominent problem in low income communities and causes more difficulties in control and treatment of bacterial diarrhea in these areas where the GI infections are yet a leading cause of mortality and morbidity.

Excessive and irrational use of antibiotics for diarrheal diseases also forces the selection of resistant GI commensals, which can serve as reservoirs of resistance genes.[9] Several studies demonstrated that resistant non-pathogenic *Escherichia coli* were more commonly carried by people in developing countries and that the trends of resistance in these bacteria paralleled that of enteric pathogens.[4] Resistance of *E. coli* spp.

Address for correspondence: Dr. Shervin Ghaffari Hoseini, Infectious Diseases and Tropical Medicine Research Center, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: shghaffari@yahoo.com
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to antibiotics complicates treatment of serious infections such as urinary tract infections and septicemia due to these pathogens as well.

In our country Iran, extensive prescription of novel and expensive antibiotics by physicians possibly leads to different antimicrobial resistant patterns in GI bacteria. Thus, close monitoring of drug susceptibility of enteric bacteria is of considerable value both for development of local treatment guidelines and for warning of national health system about this important issue.

MATERIALS AND METHODS

Setting

During summer 2013, an unusual increase in number of diarrheal diseases was noticed in Isfahan province. According to the guideline on National Surveillance System for Foodborn Diseases,[10] random fecal samples from patients with acute diarrhea were obtained and tested in laboratories of local health centers of Isfahan province. Samples were obtained from patients (with or without dysentery) in first 2-3 days of illness, before initiation of antibiotic therapy. Demographic data from patients were recorded in specific forms. Fecal samples suspicious of *Shigella* or cultures from positive samples were sent to a referral laboratory of provincial health chancellery. Detailed identification and antibiotic susceptibility tests were performed in referral lab. The project was approved by the Ethical Committee of Isfahan University of Medical Sciences, Isfahan, Iran

Microbiology

Fecal specimens were sent to referral lab in Cary-Blair transport medium (Merck, Germany), in cold boxes within 24 h. All specimens were cultured on MacConkey's agar and on more specific media: Xylose-lysine-deoxycholate, deoxycholate citrate agar, and sorbitol MacConkey agar (for isolation of *E. coli* O_{157} H_7) all kept in 37°C for 24 h. The *Shigella* isolates were identified by biochemical characterization and by culturing on differential media such as Kliger's iron agar, Simmon's citrate agar, and lysine iron agar (all media from Merck, Germany). Serotyping of *Shigella* isolates was carried out using group specific anti-sera (Mast, UK). Also specific anti O_{157} and anti H_7 (Mast, UK) were used to identify *E. coli* O_{157} H_7.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the disc diffusion method (Kirby-Bauer) according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[11] Commercially prepared and dehydrated antibiotic discs (Padtan Teb, Iran) used in this study are as follows: Co-trimoxazole (trimethoprim-sulfamethoxazole) (1.25-23.75 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg), ceftriaxone (30 μg), cefixime (5 μg), azithromycin (15 μg), and furazolidone (100 μg).

Zones of inhibition were interpreted according to the CLSI criteria[11] and classified as sensitive (S), intermediate (I), or resistant (R). The latest zone diameter interpretative criteria for ceftriaxone was used in this study (R: ≤19, I: 20-22, S: ≥23).[11] *E. coli* ATCC 25922 was used as reference strain for quality control.

For determination of multidrug-resistant isolates, tested antibiotics were classified in four groups according to the CLSI guidelines:[11] Folate pathway inhibitors (co-trimoxazole), cephems (ceftriaxone, cefixime), macrolides (azithromycin), and quinolones (ciprofloxacin, ofloxacin, and nalidixic acid).

Statistical analysis

Data were processed using SPSS-PC version 16.0 (SPSS Inc., Chicago, IL, USA). Variation of antibiotic susceptibility by type of the bacteria was assessed by Fisher's exact test according to sparse data. $P < 0.05$ were considered to be statistically significant.

RESULTS

A total of 1086 specimens were randomly obtained from patients and tested in local labs. From those, 58 samples suspicious of *Shigella* were referred to the central lab and were assessed specifically. From those, 26 were identified as *Shigella sonnei*, three as *Shigella flexneri*, one as *Shigella dysenteriae*, 25 as *E. coli* strains (10 were identified as *E. coli* inactive), one as *Pseudomonas aeruginosa*, one as *Citrobacter freundii*, and one sample was culture negative. No case of *E. coli* O_{157} H_7 was found. The specimens were submitted from at least 20 local health centers over Isfahan province from both rural and urban areas. Age of the patients ranged from 2 to 70. Nearly 21% of the patients were under 5-year-old, 41.5% were between 5 and 20, and 37.5% were more than 20-year-old. Age of the patients was not related to the type of isolated bacteria ($P = 0.124$).

Results of antibiotic susceptibility testing are summarized in Table 1. A high proportion of the isolates were resistant to co-trimoxazole (*Shigella* spp: 100%, *E. coli*: 80%), ceftriaxone (*Shigella* spp: 88.9%, *E. coli:* 56.0%), cefixime (*Shigella* spp: 85.2, *E. coli*: 68.0%), and azithromycin (*Shigella* spp: 70.4%, *E. coli*: 44.0%). Moderate resistance was detected to nalidixic acid (*Shigella* spp: 29.6%, *E. coli*: 56.0%), furazolidone (*Shigella* spp: 33.3%, *E. coli*: 12.0%), ciprofloxacin (*Shigella* spp: 14.8%, *E. coli*: 20.0%), and ofloxacin (*Shigella* spp: 25.9%, *E. coli*: 24.0%).

Among *Shigella* isolates no sensitive case to ceftriaxone was found, and only one isolate was sensitive to cefixime. Moreover, only 10 *Shigella* isolates (37%) were sensitive to ciprofloxacin.

Pattern of susceptibility (proportion of R, I, and S) for all of the tested antibiotics was significantly related to the type of the isolated bacteria, except for co-trimoxazole and ofloxacin. *E. coli* isolates were less frequently resistant to all the antibiotics in comparison with *Shigella* isolates, except for nalidixic acid and ciprofloxacin [Table 1].

About 88.3% of *S. sonnei* isolates, one *S. flexneri* isolate, and 56% of *E. coli* strains were resistant to at least three antibiotic classes (multidrug resistant) [Table 2].

DISCUSSION

Bacillary dysentery is a world-wide problem, which its incidence typically increases during the summer and also in outbreaks due to food or water source contaminations.

Most of outbreaks in African and Asian countries are due to *S. flexneri* or *S. dysenteriae*. Another important etiology of dysentery outbreaks is *E. coli* O_{157} H_7. However, in the current study in setting of a prolonged outbreak of diarrheal diseases, most frequently isolated Shigella spp. was *S. sonnei* (26/30).

S. sonnei infection is prevalent in developed countries. Historically, *S. flexneri* has been the dominant etiologic agent of dysentery in the developing world, but *S. sonnei* is now replacing it and is emerging as a problem in developing areas undergoing public health development and improvements in water quality. Recently, drug resistant *S. sonnei* strains have been more frequently detected in developing areas such as Vietnam,[12] Thailand,[3] Bangladesh,[13] and China.[14] Reports from different regions of Iran such as Tehran and Shiraz also show a tendency to increased incidence of *S. sonnei* strains, which are in concordance with our results.[15-17]

Resistance to antimicrobials is increasing throughout the world, but it is more extensive in developing countries.[4] In the current study, nearly all of *Shigella* isolates were resistant to co-trimoxazole, ceftriaxone, and cefixime, and only one sensitive *Shigella* isolate to azithromycin was found (70.4%: R, 25.9%: I). Isolated *E. coli* strains were, to a lesser extent, highly resistant to these antibiotics. Moreover resistance to ciprofloxacin and ofloxacin was unacceptably high among *Shigella* isolates (about 15% and 26% respectively). In a similar study, in Isfahan province which was done in autumn 2006, resistance to co-trimoxazole, ceftriaxone, and nalidixic acid were 85%, 33%, and 28%, respectively, and no resistance to ciprofloxacin was reported.[18] An apparent increase in resistance to co-trimoxazole, ceftriaxone, and ciprofloxacin is noted in the current study; however resistance to nalidixic acid was unchanged. Comparison of our results with another study in Isfahan (2006) on Shiga toxin-producing *E. coli* isolates also notifies an increase of

Table 1: Antibiotic susceptibility profile of *Shigella Spp.* and *Escherichia coli* strains isolated in Isfahan province during summer 2013

Antibiotic	Type of bacteria			Total (%)	*P* value*
	S. sonnei (%)	*S. flexneri* (%)	*E. coli* (%)		
Co-trimoxazole**					
R	24 (100.0)	3 (100.0)	20 (80.0)	47 (90.4)	0.085
S	0 (0.0)	0 (0.0)	5 (20.0)	5 (9.6)	
Nalidixic acid					
R	7 (29.2)	1 (33.3)	14 (56.0)	22 (42.3)	0.000
I	17 (70.8)	0 (0.0)	4 (16.0)	21 (40.4)	
S	0 (0.0)	2 (66.7)	7 (28.0)	9 (17.3)	
Ciprofloxacin					
R	3 (12.5)	1 (33.3)	5 (20.0)	9 (17.3)	0.002
I	13 (54.2)	0 (0.0)	2 (8.0)	15 (28.8)	
S	8 (33.3)	2 (66.7)	18 (72.0)	28 (53.8)	
Ofloxacin					
R	5 (20.8)	2 (66.7)	6 (24.0)	13 (25.0)	0.194
I	8 (33.3)	0 (0.0)	3 (12.0)	11 (21.2)	
S	11 (45.8)	1 (33.3)	16 (64.0)	28 (53.8)	
Ceftriaxone					
R	24 (100.0)	0 (0.0)	14 (56.0)	38 (73.1)	0.000
I	0 (0.0)	3 (100.0)	4 (16.0)	7 (13.5)	
S	0 (0.0)	0 (0.0)	7 (28.0)	7 (13.5)	
Cefixime					
R	23 (95.8)	0 (0.0)	17 (68.0)	40 (76.9)	0.001
I	1 (4.2)	1 (33.3)	5 (20.0)	7 (13.5)	
S	0 (0.0)	2 (66.7)	3 (12.0)	5 (9.6)	
Azithromycin					
R	17 (70.8)	2 (66.7)	11 (44.0)	30 (57.7)	0.043
I	7 (29.2)	0 (0.0)	9 (36.0)	16 (30.8)	
S	0 (0.0)	1 (33.3)	5 (20.0)	6 (11.5)	
Furazolidone					
R	7 (29.2)	2 (66.7)	3 (12.0)	12 (23.1)	0.006
I	15 (62.5)	1 (33.3)	10 (40.0)	26 (50.0)	
S	2 (8.3)	0 (0.0)	12 (48.0)	14 (26.9)	

*Fisher exact test; **No. case of intermediate susceptibility was detected for co-trimoxazole; R = Resistant; I = Intermediate susceptibility; S: Sensitive; *E. coli = Escherichia coli*; *S. sonnei = Shigella sonnei*; *S. flexneri = Shigella flexneri*

Table 2: Resistance pattern of isolated pathogens from patients suspicious of shigellosis in Isfahan

Type of bacteria	Number of antibiotic classes					Total
	0 (%)	1 (%)	2 (%)	3 (%)	4 (%)	
S. sonnei	0	0	4 (16.7)	11 (45.8)	9 (37.5)	24
S. flexneri	0	0	2 (66.6)	1 (33.3)	0	3
E. coli	2 (8.0)	5 (20.0)	4 (16.0)	5 (20.0)	9 (36.0)	25
Total	2 (3.8)	5 (9.6)	10 (19.2)	17 (32.7)	18 (34.6)	52

E. coli = Escherichia coli; *S. sonnei = Shigella sonnei*; *S. flexneri = Shigella flexneri*

resistance to ceftriaxone, ciprofloxacin, and ofloxacin in these bacteria.[19]

Several studies from Iran which all were carried out before 2006 reported low resistance to ciprofloxacin, ceftriaxone, and cefixime (all lower than 10%).[15,20,21]

Different patterns of antibiotic resistance is seen in various regions of the world, for example more than 80% of *Shigella* isolates were resistant to nalidixic acid in Bangladesh,[13] China,[14] and India.[22] According to the 2010 annual report of National Antimicrobial Resistance Monitoring system in USA,[8] rate of resistance of *Shigella* isolates to nalidixic acid and ciprofloxacin was 4.4% and 1.7%, respectively, and less than 1% of isolates were resistant to cephems.[8]

Differences in laboratory methods might stand for some of disagreements between results of similar studies, however resistance to most of tested antibiotics were obviously high in the current study. Reasons for high antimicrobial resistance in our region are probably irrational prescription of antibiotics by physicians, over-the-counter use of antibiotics by patients, and massive use of antibiotics for treatment or prophylaxis in food animals and agriculture.[4] High resistance to expensive and relatively new antibiotics in our region (such as azithromycin, ceftriaxone, and cefixime) is indeed the result of misuse of these antibiotics in human diseases, as these agents are not utilized in agriculture.

Resistant to ciprofloxacin is an important issue universally because it is the first choice not only for empirical therapy of dysentery in adults but also for severely ill children unresponsive to other drugs.[6] Furthermore, Azithromycin and cefixime are first recommendations for dysentery in children under 18.[23] We detected a sever decrease in susceptibility of enteric pathogens to first line treatment choices of dysentery and indeed this is a warning for national health system.

Some limitations are noted in this study: It is suggested to use more accurate dilution methods or E-tests for susceptibility testing, and to design a systematic random sampling in future studies, although it was a community based research in contrast to many previous studies, which obtained samples from hospitalized patients.

CONCLUSION

Development of a national integrated surveillance system for monitoring antibiotic resistance seems mandatory in our country. This system has to continuously monitor drug susceptibility of important pathogens such as etiologic agents of enteric and respiratory infections, as well as close control of antibiotic prescription by physicians and its use in agriculture. Considering the trend of drug resistance in Iran, it seems that soon we will have intense difficulties for treatment of severely ill patients who truly need antibiotics, especially children and immunocompromised patients.

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AUTHORS' CONTRIBUTION

All authors have contributed in designing and conducting the study. AFS, AA, MZ, ES, and MS collected the data and RF, AFS, BA, and SGH did the analysis. All authors have assisted in preparation of the first draft of the manuscript or revising it critically for important intellectual content. All authors have read and approved the content of the manuscript and are accountable for all aspects of the work.

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