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Resistance of *Shigella* strains to extended-spectrum cephalosporins in Isfahan province

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Abstract

Background: The aim of this study was to determine the serotypes and antimicrobial susceptibility of *Shigella* spp. in Isfahan, (Iran) from 2010 to 2015.

Methods: This retrospective study was conducted on *Shigella* isolates in four tertiary care hospitals. The process of bacterial isolation and determination of susceptibility was performed by standard microbiological guidelines. The patients were categorized into three age groups of under 5, 5-15 and over 15 years.

Results: Among 45 isolates, *S. sonnei* (63.6%) was the predominant species, followed by *S. flexneri* (34.1%), and *S. dysenteriae* (2.3%). Substantial resistance to ampicillin, trimethoprim- sulfamethoxazole, ceftriaxone, cefotaxime, and cefixime was observed. Over 94% of the isolates were sensitive to ciprofloxacin. Susceptibility of isolates was similar between all age groups.

Conclusion: Significant resistance to third generation cephalosporins precludes the use of these agents for empirical treatment of shigellosis in our population. Ciprofloxacin is an appropriate option; however, susceptibility tests should be performed before prescription.

Keywords: Shigellosis, Susceptibility, Ciprofloxacin, Third Generation Cephalosporin, Iran.

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Introduction

Shigellosis is a universal cause of enteric bacterial infection with sporadic outbreaks and epidemics (1). Recently, a regional outbreak had been occurred in our province (2). Antibacterial therapy reduces not only the duration and severity of the infection, but also the fecal elimination of the organism, which in turn would preclude its further spread (3). However, the bacterium rapidly develops resistance to frequently prescribed antibiotics (4).

In accordance to the World Health Organization (WHO) guidelines, the choice of treatment should be selected by the antimicrobial susceptibility of locally circulating *Shigella* strains (4).

Inappropriate utilization of antibiotics for nonbacterial illnesses, especially in developing countries (5), forces the selection of resistant *Shigella strains* (6). Widespread resistance to ampicillin, trimethoprim- sulfamethoxazole, and nalidixic acid during the past years has made these antibactrials to be no longer beneficial for empirical treatment in many parts of the world [4]. Furthermore, resistance to ceftriaxone and ciprofloxacin has been reported in Southeast Asia, and to a lesser extent in some areas of Iran (7-9).

Despite the presence of some reports in Iran about the susceptibility pattern of *Shigella strains* (9-16), there is no recent report about the sensitivity of ongoing strains

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in Isfahan province. During the summer of 2013, an outbreak of shigellosis was noticed in Isfahan province, which would not be representative of circulating bacteria in the area (2). Therefore, this study aimed to identify the antimicrobial susceptibility of *Shigella strains* isolated in high- quality laboratories in our region to support selecting a suitable antimicrobial agent for empiric treatment of *Shigella* cases.

Methods

Subjects: This study was conducted among the culture-proven *Shigella* cases that were admitted to four large referral hospitals in Isfahan province (Alzahra, Shariati, Gharazi, and Fateme-Zahra Najaf-Abad medical centers) from April 2010 to April 2015. The medical laboratories of these tertiary care general centers attained the WHO standard approval in microbiological reports. Studied isolates were processed as a part of standard patient care.

Demographic information of the patients was reviewed, and information on each isolate was collected and recorded with respect to its serotype and antimicrobial susceptibility profile. The patients were categorized into three age groups of less than 5, 5-15 and over 15 years.

Shigella Identification: The bacterial isolation was conducted by conventional tests (oxidase, Indole, Methyl red, Voges-Proskauer, Citrate, Triple sugar Iron agar, Motility, Hekton agar). (Pronabisa, Spain) (17). *Shigella* serotypes were identified by type specific anti-sera (Pronabisa, Spain) (17).

Antimicrobial Susceptibility Test: Antibiotic susceptibility testing was done using the disc diffusion technique (Kirby-Bauer) in accordance to Clinical and Laboratory Standards Institute (CLSI) guidelines (18). Commercially prepared and dehydrated antibiotic discs (Mast, UK) applied in the study were as follows: trimethoprim- sulfamethoxazole (1.25/23.75µg), ciprofloxacin (5µg), ampicillin (10µg), ceftriaxone (30µg), cefixime (30µg), cefotaxime (30µg), piperacillin-tazobactam(100/10µg), and meropenem (10µg).

Ethical Consideration: The study protocol was reviewed and approved by the Institutional Review Board of Isfahan University of Medical Sciences. Because the focus was on bacteria, it was not necessary to obtain patients' consent.

Statistical Analysis

Data were processed using SPSS-PC Version 18.0 (SPSS Inc., Chicago, IL, USA). Variation of antibiotic susceptibility was assessed by serotype of the bacteria and by age groups, using Chi- square test according to sparse data. $P<0.05$ was considered as statistically significant.

Results

Overall, 45 *Shigella* cases were identified (21 male, 24 female). The mean±SD age of the participants was 20.4± 24.06 years. Thirteen (28.9%) individuals were under 5 years of age, 16 (35.6%) between 5 and 15 and 16 (35.6%) above 15. *Shigella sonei* was the commonest serotype (63.6%) followed by *S. flexeneri* (34.1%) and *S. dysentery* (2.3%). Distribution of serotypes was similar between the age groups (Table 1).

Among antibiotics tested for susceptibility of *shigella* strains, ciprofloxacin was the best with 94.9% sensitivity followed by cefotaxime (43.8% sensitivity). Less than 30% of *Shigella strains* were sensitive to ceftriaxone, cefixime, ampicillin, and trimethoprim- sulfametoxazole (Table 2). Susceptibility of *Shigella strains* was simi-

Table 1. Frequency Distribution of *Shigella* Serotypes in Different Age Groups, Isfahan, Iran, 2010-2015

Shigella Serotype	Age Group (yrs)				p
	< 5	5-15	>15	Total	
Sonei	9(75.0)*	9(56.3)	10(62.5)	28(63.6)	
Flexeneri	3(25.0)	7(43.7)	5(31.3)	15(34.1)	0.575
Dysentry	0	0	1(6.2)	1(2.3)	
Total	12(100.0)	16(100.0)	16(100.0)	44(100.0)	

Table 2. Antimicrobial Drug Sensitivity of *Shigella* spp., Isfahan, Iran, 2010–2015

Antibiotic medication	Sensitivity of *Shigella* sp., no. (%) isolates				
	S. sonei	S. flexeneri	S. dysentry	Total	p
Ciprofloxacin	23/25(92.0)	13/13 (100)	1/1 (100)	37/39 (94.9)	0.554
Cefotaxime	8/17 (47.1)	5/13 (38.5)	0/1 (0)	14/31 (45.2)	0.616
Ceftriaxone	5/14 (35.7)	3/12 (25.0)	0/1 (0)	8/27 (29.6)	0.673
Cefixime	6/18 (33.4)	2/12 (16.7)	0/1 (0)	8/31 (25.8)	0.496
Ampicillin	3/18 (16.7)	0/4 (0.0)	----	3/22 (13.6)	0.380
Trimetoprim/Sulfamethoxazole	3/28 (10.7)	1/12 (8.3)	0/1 (0)	4/41 (9.8)	0.921

Table 3. Results of Antibiotic Susceptibility Testing in Shigella Serotypes in Accordance to Age Groups, Isfahan, Iran, 2010-2014

Antibiotic	Sensitivity in age group: number of sensitive/number of tested(%sensitivity)				
	< 5 year	5-15 year	>15 year	Total	p
Ciprofloxacin	7/8 (87.5)	15/15 (100.0)	15/16 (93.8)	37/39 (94.9)	0.418
Cefotaxime	6/9 (66.7)	5/12 (41.7)	3/11 (27.3)	14/32 (43.8)	0.206
Ceftriaxone	2/6 (33.3)	4/11 (36.4)	2/10 (20.0)	8/27 (29.6)	0.696
Cefixime	2/7 (28.6)	4/13 (30.8)	2/11 (18.2)	8/31 (25.8)	0.768
Ampicillin	2/10 (20.0)	1/5 (20.0)	1/8 (12.5)	4/23 (17.4)	0.903
Trimetoprim-Sulfamethoxazol	1/12 (8.3)	1/15 (6.7)	1/15 (6.7)	3/42 (7.1)	0.813

lar between the three age groups (Table 3). Interestingly, all 7 isolates, which were tested for susceptibility to piperacillin-tazobactam were sensitive and all 4 specimens that were examined for sensitivity to meropenem were non susceptible.

Discussion

This study revealed the appearance of *Shigella* serotypes with high level (> 50%) of resistance to third generation cephalopsporins and very high level (>90%) to ampicillin and trimethoprim- sulfametoxazole in the region. Resistance to ciprofloxacin was low, suggesting that this fluoroquinolone could be an effective antibiotic for the treatment of acute shigellosis in our district.

Ampicillin and trimethoprim- sulfamethoxazole resistance among *Shigella strains* have been vastly reported from developing as well as developed countries (1,7,8). All studies conducted during the past 20 years in Iran showed a high and persistent rate of resistance to these two agents. Less than 30% of isolated *Shigella* species were sensitive to either of these antibiotics in Ghazvin in 2001 (10), Mashhad in 2005 (11), Zanjan in 2003-2007 (9) and Zahedan in 2004 (12). Therefore, these antibacterial medications are not appropriate for the empirical treatment of shigellosis in Iran and many other countries (1).

Low levels of resistance to ceftriaxone, cefotaxime and cefixime have been previously reported from some districts of Iran. Less than 5% of *Shigella strains* in Zahedan in 2004(12), Tehran in 1996-2005 (13), Mashhad in 2005 (11) and Tehran in 2009(14) were resistant to these antibiotics, representing them as an ideal antibiotic for treatment of *shigella* cases in these districts. About 10-20% of isolated *shigella* species in Fasa in 2005 (15) and greater than 20% in Zanjan in 2003-2007 (9) and Tehran in 2011 (16) were resistant to either of these the agents, indicating that these antibiotics would no longer be an appropriate option for complicated cases of shigellosis in those regions. In our study, greater than 50% of isolates were resistant to cefotaxime, and greater than 70% were resistant to ceftriaxone and cefixime. The rate of resistance was similar in children and adults. The emergence of strains with high levels of resistance to third generation cephalosporins, which are the cores in the treatment of shigellosis in children, is an important issue that will necessitate making a new decision for antibiotic therapy of the disease. Ciprofloxacin (or other fluoroquinolones) is commended by the WHO as the first choice for the treatment of Shigellosis in both children and adults (1). Despite the restriction of fluoroquinolone prescription in children, the first choice of an-

tibiotic in all ages in our region should be changed from third generation cephalosporin to a flouroquinolone.

The overall resistance to ciprofloxacin in our study was low (<5.0%), suggesting that fluoroquinolone is still an ideal antibacterial for treating acute shigellosis in our population; however, treatment failures due to resistant strains may occur. Studies conducted in other parts of Iran (Zahedan in 2003 (12), Tehran in 1996-2005 and 2009 (13,14), Mashhad in 2005 (11), and Fasa in 2005(15)), revealed similar results. In a study in Zanjan in 2003-2007 (9), greater than 10% of *Shigella* species were resistant to ciprofloxacin. The rate of resistance to this agent in some Southeast Asian countries such as Bangladesh and India, which have been using these agents for many years, exceeds 40% (5,16,17). The appearance of highly resistant *Shigella strains* to fluoroquinolone in these countries should be alarming for our national health system. Excessive and irrational use of antibiotics for non- bacterial illness is a common scenario in Iran as well as many other developing and developed countries (5). Failure to plan effective strategies to reduce the overall usage of antibiotics in the community can take humans to the era of no antibiotic discoveries.

Our finding in few cases that tested the susceptibility to meropenem revealed that all examined isolates were resistant to this antibacterial agent, making it a non- suitable option in resistant strains. However, piperacillin-tazobactam was effective in all the seven examined strains. In a recent study in Tehran in 2012 (16), more than 98% of the isolated *Shigella strains* were sensitive to imipenem, suggesting that regional differences in the sensitivity of the microorganism exist. We propose that studying the susceptibility of *Shigella strains* to these two agents should be evaluated in future studies.

Shigella sonei was the most common isolated serotype of *Shigella* in this study. For many years, in Asia and most developing countries including many parts of Iran, the most common isolated serotypes of *Shigella* was *S. flexeneri* (8-11,14), but reports from Tehran in 2008, 2009 and 2011 (13,14,16) showed an increase in *S. sonei* . We believe that *S. sonei* may become the most prevalent serotype of *Shigella* in industrialized counties of Iran.

Our study had some limitations. First, it was a retrospective study of laboratory records of the referees to four general hospitals; therefore, the findings cannot be generalized to the whole population. Drug resistance might be more common among *Shigella strains* isolated from hospitalized patients, leading to over-estimation of the level of resistance to some antibiotics. Secondly, the antibiotic resistance in our study was identified by disc diffusion method without assessing MIC breakpoints. The third limitation was the study sample size that seems to be inadequate for certain conclusions. Despite these limitations, the findings of our study revealed important changes in the epidemiology and appearance of *Shigella strains* with the high level of resistance to third generation cephalosporins in our district. These results could certainly help the physicians to employ the best choices to treat shigellosis in the region.

Conclusion

In conclusion, our study revealed an epidemiological shift in *Shigella strains* with progressive increase in *S. sonnei* in our population. Substantial increase in resistance to the third generation cephalosporin in all age groups precludes the use of these agents for empiric use of these agents for the treatment of *Shigella* infection in our population. This is a warning for health care agencies to implement policies for the prudent use of antibiotics. Fluoroquinolone is an effective line option; however, sensitivity testing should be done before prescription.

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