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Antimicrobial Resistance of *Shigella* from Patients with Acute Diarrhea. Quaemshahr, Mazandaran, Iran

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Abstract: This study was performed to determine the pattern of antimicrobial resistance of *Shigella* sp. in patients with acute diarrhea. The study included all patients with acute diarrhea who were visited in a teaching hospital during 14 months period (2004- 2005). The isolates were confirmed by biochemical reaction and specific antisera. Antibiotic susceptibility test was determined by agar diffusion method. Of the 273 stool samples, 130(47.6%) yielded *Shigella* sp. *Shigella flexneri* caused 94(72.3%) of the total cases of shigellosis followed by *S. dysenteriae* 17 (13.1%), *S. boydii* 15 (11.5%) and *S. sonnei* 4 (3.1%). All 130 isolates showed resistance at least to one of eleven antibiotics and 123/130 (94.6%) were resistant to two or more antimicrobial agents. The high number of multi-drug resistance isolates and decreased susceptibility to main antibiotics for treatment of *Shigella* sp. warrant need to continuous monitoring of antimicrobial susceptibilities through a surveillance system for effective therapy and control measures.

Key words: Diarrhea, antimicrobial resistance, *Shigella*, patients, surveillance

INTRODUCTION

Infection with a *Shigella* sp. was an essential cause of morbidity and public health concern in Iran and other developing countries, but it is sporadic in industrialized countries (Lima *et al*., 1995; Gaikwad and Deodhar, 1985; Daniel *et al*., 2004).

Shigella sp. remained an important cause of gastrointestinal illness manifested by watery diarrhea, which may progress to mucoid and bloody diarrhea (Hirose *et al*., 2005; Kotloff *et al*. 1999). The annual number of *Shigella* episodes throughout the world was estimated to be 164.7 million, of which 163.2 million were in developing countries with 1.1 million deaths (Ashkenazi, 2004). *Shigella* sp. are capable of causing illness vary from mild self-limited to severe bloody diarrhea especially in children, the elderly, the malnourished and the immunocompromised individuals who need administration of an effective antimicrobial agent as soon as the clinical diagnosis is made (Cheasty *et al*., 2004; Marilyn *et al*., 2000; Swapan and Niyogi, 2005).

Studies have reported high rates of resistance of *Shigella* sp. to variety of antibiotics, specially to ampicillin, Trimethoprim-Sulfamethoxazole (TMP-SMZ), nalidixic acid, tetracycline and erythromycin (Lima *et al*., 1995; Cheasty *et al*., 2004; Marilyn *et al*., 2000; Swapan and Niyogi, 2005).

The worldwide increasing antimicrobial resistance of *Shigella* is a major concern. A regional study (Zaly *et al*., 2003; Hoseini *et al*., 2002) in Iran showed high rate of resistance of *Shigella* sp. to ampicillin, tetracycline, TMP-SMZ and variable resistant to other antimicrobial agents. The current study was therefore, conducted to determine the rate of *Shigella* isolates from stool sample that was collected from patients with acute diarrhea who referred to infectious disease and pediatrics departments of a teaching hospital in Mazandaran province in north of Iran, to verify predominant serotypes of *Shigella* sp., antibiotic susceptibility pattern and to evaluate if continuous local monitoring of antimicrobial susceptibility pattern of *Shigella* isolates is needed.

MATERIALS AND METHODS

The present study was conducted in infectious disease and pediatrics departments of a teaching hospital in Quaemshahr, the main referral center of infectious diseases of Mazandaran province-Iran, during a period of 14 months from September 2004 to November 2005.

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Participants were all patients with acute diarrhea who presented to emergency department or outpatient clinics of the Razi teaching hospital. Acute diarrhea was defined as the passage of 3 or more liquid stools with or without blood and mucus in a 24 h period. Multi-drug resistance defined as resistance to 2 or more of 11 selected antimicrobial agents.

Bacteriological analysis: Stool specimens were collected with a cotton swab, transported to the laboratory in buffer glycerol phosphate medium. Selective and differential media were used for isolating *Shigella* sp. The samples were primarily cultured on *Shigella-salmonella*, MacConkey agar media and (Xylose-Lysine-Desoxycholate) XLD Agar (Merck KGaA-Darmstadt-Germany). All plates were incubated aerobically at 37°C overnight. The Non-Lactose-Fermenting (NLF) colonies were identified on urea hydrolysis, Triple Sugar Iron (TSI) medium, Sulphide-indole and Motility Medium (SIM) and Simmon's citrate test. They were further identified at group level by slide agglutination test with specific antisera [Bahar afshan company-Tehran-Iran].

Antimicrobial susceptibility testing: Resistance patterns of the *Shigella* isolates to various antibiotics were determined by the agar diffusion technique. Every inoculum was prepared by inoculating 5 mL of Mueller-Hinton broth with five colonies of an 18 h old pure *Shigella* culture followed by incubation in ambient air and at 37°C for 16 h. The resulting turbid culture was standardized to a turbidity of 0.5 McFarland using 0.85% NaCl as a diluent. A sterile cotton swab was dipped into the standardized suspension, drained and used for inoculating 25 mL of Mueller-Hinton Agar (MHA) in a 90 mm plate. The inoculating plates were air dried and antibiotic disks included ampicillin (10 μg), chloramphenicol (30 μg), erythromycin (15 μg), nalidixic acid (30 μg), cefixime (5 μg), ciprofloxacin (5 μg), TMP-SMX (1.25/23.75 μg), ceftriaxone (30 μg), gentamicin (10 μg), tetracycline (30 μg) and amoxi-clavulante (20/10 μg) [Padtan Teb-Tehran_Iran]. Standard control strains were used for monitoring the accuracy and exactness of the disk diffusion test. The interpretation of zone diameters inhibition was that recommended by NCCLS (2000).

For analysis of data, SPSS version 12.0 was used. The significance of proportional differences between nominal variables was determined using the chi-square test or Fisher exact test and differences between continuous variables were determined using student t-test. A two-tailed $p<0.05$ was used to define statistical significance.

RESULTS

Shigella sp. was isolated from 130 (47.61%) of 273 stool samples. Of these, *Shigella flexneri* was the most common isolate in all age groups 94 (72.3 %), followed by *S. dysenteriae* 17 (13.1 %), *S. boydii* 15 (11.5%) and *S. sonnei* 4 (3.1%). Overall isolation rate of *Shigella* was observed to be more among children

Table 1: Rates of resistance reported for *Shigella* sp.

	Resistance (%)				
Antimicrobial agent	All isolates N = 130	*S. flexneri* N = 94	*S. dysenteriae* N = 17	*S. boydii* N = 15	*S. sonnei* N = 4
Amoxicillin-Clavulanate	54.6	25.5	41.2	46.5	75
Ampicillin	31.5	54.3	64.7	40.0	75
Cefixime	16.2	13.8	23.5	20.0	25
Ceftriaxone	6.9	4.3	17.6	6.7	25
Chloramphenical	26.2	25.5	23.5	26.7	50
Ciprofloxacin	6.2	5.3	11.7	6.7	0
Erythromycin	54.6	50.0	70.5	60.0	75
Gentamicin	6.9	6.4	5.8	6.7	25
Nalidixic acid	41.5	45.5	41.2	33.3	50
Tetracycline	50.0	46.8	52..9	53.3	100
TMP-SMZ	59.2	54.3	70.5	80.0	50

Table 2: Rates of *Shigella* isolates with multi-drug resistance

	Resistance (%)				
Antimicrobial agents	*S. Flexneri* N = (%)	*S. dysenteriae* N = (%)	*S. boydii* N = (%)	*S. sonnei* N = (%)	All species N = (%)
TMP-SMZ+Ery	28.7	47.0	53.3	50	34.6
TMP-SMZ+Tet	29.6	41.0	53.3	50	34.4
Ery+Tet	28.7	53.0	40.0	75	34.4
Amp+TMP-SMZ	35.3	59.0	40.0	50	39.4
Amp+Ery	29.7	41.0	33.3	75	33.0
Amp+TMP-SMZ+Ery	21.0	35.3	33.3	50	25.1
Amp+TMP-SMZ+Ery+Tet	17.1	35.3	26.6	50	21.5
Amp+TMP-SMZ+Ery+tet+NA	9.6	6.0	20.0	50	11.5

5 years old and below 34/130 (26.15%), followed by 6-15 years 22/130 (16.9%) and adults 65 years and over15/130 (11.5%). Seventy patients (53.8%) were male and 60 (46.2%) were female. Detection rates was highest in summer [June-September] 68/130 (52.3%), moderate in autumn [October-November] 46/130 (35.4%) [Including autumn of year 2003 (29/130,) and 2004 (17/130] and lowest in winter and spring 8/130(6.2%) each. All 130 isolates were resistant to at least one of 11 antibiotics. Resistance to TMP-SMZ was highest 77/130(59.2%); more than half of microbial isolates were resistant to one of the following antimicrobials: Amoxicillin-clavulanate, erythromycin, tetracycline and TMP-SMZ. Significant number of isolates was resistant to nalidixic acid and nearly one third to ampicillin. Even there were number of isolates resistant to ceftriaxone and ciprofloxacin, actually none of the isolates were fully sensitive to any antimicrobial agents that tested for (Table 1).

In 130 isolates, 8 patterns of antibiotic resistance were found, which on further analysis revealed that nearly 123/130 (94.6%) isolates were resistant to 2 or more antimicrobial agents (Table 2).

DISCUSSION

In current study, the overall frequency of *Shigella* isolates among patients with acute diarrhea who referred to Razi hospital was more than 45%. This is a very high rate of *Shigella* isolates from stool samples of patients with acute diarrhea in comparing to other regional studies (Al-Gallas *et al*., 2007). In a recent study of etiology of acute diarrhea in children and adults in Tunis showed that the majority of isolates with acute diarrhea were *E. coli*, *adenovirus* and *salmonella*. *Shigella* sp. were isolated only in 4% of samples from adults with acute diarrhea. In a study in Ghana (Opintan and Newman, 2007) from a teaching hospital they noted out of 594 diarrhea stool specimens 24 *Shigella* sp. were isolated which denotes an isolation rate of 4%. However, in a study (Zaly *et al*., 2003) of 734 stool samples with acute diarrhea in Karaj-Iran they isolated 123 (16.7%) *Shigella* sp., which indicates a higher proportion of *Shigella* isolates from two studies carried out in two different regions of our country in comparing to two other studies. We indicated that *S. flexneri* was the predominant serogroup followed by *S. dysenteriae*, *S. bodii* and *S. sonnei*. The distribution of sp. varies from one country to another country and in different regions of the same country. *S. sonnei* was the most predominant species in USA, European countries and Canada (Marilyn *et al*., 2000; Dupont, 2000; Onder, 2004), whereas *S. flexneri* was more common in developing countries (AL-Eisa *et al*., 1992; Zaman *et al*., 1991).

The emergence and dissemination of antimicrobial resistance among *Shigella* strains is an increasing global health problem that is complicating the therapeutic management of severe shigellosis cases. Studies (Yamashiro *et al*., 1998; Gascon *et al*., 2000; Youssef *et al*., 2000; Bennish *et al*., 1992; Bogaerts *et al*., 1997) from many regions of world have already reported an increase in isolation of *Shigella* sp. that were resistant or exhibiting multidrug-resistance to clinically important and commonly used antimicrobial agents. Our findings are steady with those reports in that we also observed an alarmingly high prevalence of *Shigella* that were resistant to antibiotics frequently are available and used for treating shigellosis by clinicians in Iran.

Comparable with other studies (Mamun *et al*., 1997; Hoge *et al*., 1998; Ashkenazi *et al*., 2003). Our results confirmed the high prevalence of resistance to tetracycline and erythromycin in *S. flexneri*, despite the fact that these antimicrobials have not been used as a common antibiotic for treatment of suspected cases of shigellosis during recent years in Iran and that tetracycline is not used for any kind of infection in children. The persistence of TMP-SMX, ampicillin resistance may be explained by the extensive use of these agents for treatment of various infections in Iranian children, causing selection of resistant strains. It is also noteworthy, that in some studies (Fulla *et al*., 2005) none of the *Shigella* strains were resistant to nalidixic acid and ciprofloxacin, however we observed a high rate of resistance toward nalidixic acid. In a study in Karaj-Iran (Zaly *et al*., 2003) cefixime or cetriaxone offered as the drug of choice in the treatment of shigellosis in children and nalidixic acid for adults based on antimicrobial susceptibility patterns. While current study demonstrates more than 40% of *Shigella* sp. are resistance to nalidixic acid and quite a significant number to cefixime and ceftriaxone. Resistance to TMP-SMX or to both ampicillin and TMP-SMX was more common among *S. flexneri* and *S. dysenteriae*, none of these species were pan-sensitive. The proportion of isolates resistant to ampicillin, TMP-SMX, tetracycline and erythromycin is more than 50% and over 40% to nalidixic acid, additionally some isolates were multi-resistant to ampicillin, TMP-SMX, tetracycline, erythromycin and nalidixic acid which indicates these agents are no longer appropriate for empirical treatment in this part of the country. Nalidixic acid was effective and still is approved for treatment of shigellosis in children older than 3 months in many countries (Dupont, 2000; Bennish *et al*., 1992; Salam and Bennish, 1991) but reports of antimicrobial resistance trends in *Shigella* isolates from some countries raise the presence of wider resistance to nalidixic acid, fluoroquinolones and ceftriaxone in the future (Bennish *et al*., 1992; Bogaerts *et al*., 1997; Ashkenazi *et al*., 2003).

In this study, quite a few *Shigella* isolates were resistant to ceftriaxone or ciprofloxacin, while these agents may offer reliable results when used for empirical treatment of shigellosis, clinicians should expect increasing resistance to them as their use increases. The high prevalence of antimicrobial resistance among *Shigella* isolates noted in this study limits safe and effective treatment opportunities for shigellosis principally in children. Where resistance to ampicillin, nalidixic acid and TMP-SMX is common, appropriate antimicrobial agents for the treatment of shigellosis are limited to ceftriaxone, fluoroquinolones, or azithromycin. Although the broader-spectrum fluoroquinolones are not accepted for use in children, because they have been shown to cause cartilage damage in young animals, several studies (Chusky and Hullman, 1992; Zimbabwe, Bangladesh, South Africa [Zimbasa] 2002; Leibovitz *et al*., 2000) showed that a short course of ciprofloxacin therapy in pediatric patients with particular enteric infections could be a universal practice among pediatricians and infectious disease specialists without the development of joint abnormalities. Another antimicrobial agent that remained highly efficient against *Shigella* was the third-generation cephalosporins, which is appropriate for severe systemic infections or for clinically severe shigellosis caused by multi-resistant strains. The strength of the current study is that this is the first study in our country that showed the very high resistance of *shigella* sp. to different antimicrobials, particularly to nalidixic acid which supposed to be the antibiotic of choice in the treatment of shigellosis in children.

CONCLUSION

In conclusion *Shigellae* sp. are predominant organisms in acute gastroenteritis patients who referred to this teaching hospital. Although this study and previous regional studies may not indicate the whole picture of the country profile it may indicate that the emergence of multiple drug resistance microorganisms demands continuous monitoring of the susceptibility pattern of *Shigella* isolates. For areas where shigellosis is endemic, recommendations on antibiotic selection must be periodically updated depending on surveillance of antimicrobial resistance pattern.

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