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ANTIMICROBIAL RESISTANCE OF SHIGELLA SPP. ISOLATED FROM DIARRHEAL PATIENTS IN ZAHEDAN

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Abstract- One of the great challenges in the treatment of infectious diseases is the resistance of pathogenic bacteria against antibiotics, and antibiotic resistance to *Shigella* is broadly observed in different parts of the world. The object of this study was to determine *Shigella* antibiotic resistance pattern against the antibiotics such as ampicillin, amoxicillin, trimethoprim-sulfamethoxazole, chloramphenicol, nalidixic acid, ciprofloxacin and ceftriaxone. In this cross-sectional study, a total of 147 *Shigella* strains were collected from the diarrheic patients referring to different medical centers of Zahedan. Specific antisera were used for serotyping of isolated *Shigella* and their antibiotic resistance patterns were determined by standard Kirby-Bauer method. Of the 147 studied *Shigella* strains, 102 (69.3%) belonged to *S. flexneri*, 32 (21.7 %) to *S. dysenteriae*, 11 (7.4%) to *S. boydii*, and 2 (1.36%) to *S. sonnei* species. The isolated strains showed resistance to ampicillin (99.3%), trimethoprim-sulfamethoxazole (52%) and nalidixic acid (1.3%), but there was no resistance against ciprofloxacin and ceftriaxone. According to the findings, it is suggested that antibiotics should not be used without laboratory testing (antibiogram).

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Key words: *Shigella*, antimicrobial resistance, antibiotic, diarrhea

INTRODUCTION

Shigellosis is an acute gastroenteritis which is one of the most common causes of morbidity and mortality in children with diarrhea in developing countries. It is caused by microorganisms belonging to the genus *Shigella*. The annual number of *Shigella* episodes throughout the world is estimated to be 164.7 million, with 69% of all deaths attributable to shigellosis involving children less than 5 years old (1).

The disease is highly contagious due to its low infectious dose (2). Epidemics usually occur in areas with crowding and poor sanitary conditions (3-6). Essential events in the pathogenesis of *Shigella* infections include bacterial invasion of epithelial cells, escape from the phagosome, and induction of apoptosis in macrophages (7). Shigellosis is caused by any of the four species of *Shigella*, namely, *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. Except for *S. sonnei*, each species contains multiple serotypes, based on the structure of the O antigen (8).

More than 140 million cases of Shigellosis are reported annually worldwide and it is responsible for death of 6 million children under the age of 5, especially in developing countries. The symptoms of

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Shigellosis might be mild to serious and apart from general and intestinal symptoms, there may be other complications such as hemolytic uremic syndrome. In this syndrome, 50% of the patients suffer from serious kidney dysfunction and 5 to 10% of them expire (9).

The epidemiology and antibiotic susceptibility of shigella species change. Antibiotic resistance is seen as increasing or decreasing in this concern (10). Therefore antibiotic resistance pattern of *Shigella* must be determined to assist the doctors to choose the suitable antibiotics.

The present study was done to determine the drug resistance pattern of *Shigella* species in Zahedan, south-east of Iran.

MATERIALS AND METHODS

In this cross-sectional study, 147 *Shigella* strains were isolated from patients with bloody diarrhea in Zahedan during 2003-2004. The study was approved by Ethics Committee of Zahedan University of Medical Sciences.

The specimens were cultured on differential and selective media including XLD agar and SS agar. After 48 hours incubation in 37° C, biochemical tests were used to confirm the bacteria including growth on TSI agar, SIM, Simmons citrate and MR-VP reaction and *etc.* (11).

Specific antisera (Difco *Shigella* Antiserum) were used for serotyping of isolated *Shigella*. The susceptibilities of all isolates to different antibiotics were determined by the disk diffusion method, using amoxicillin, trimethoprim-sulfamethoxazole, ampicillin, chloramphenicol, nalidixic acid, ciprofloxacin and ceftriaxone (Bio Merieux Anti. Disk)

RESULTS

Results revealed that from 147 studied *Shigella* strains, 102 strains belonged to *S. flexneri* (69.4%), 32 strains to *S. dysenteriae* (21.8%), 11 strains to *S. boydii* (7.5%) and 2 strains to *S. sonnei* (1.3%) species (Table 1).

Resistance pattern against the applied antibiotics were as follows: ampicillin (99.3%), trimethoprim-sulfamethoxazole (57.1%), chloramphenicol (52%),

Table 1. The frequency of *Shigella spp.* isolated from diarrheic patients in Zahedan in 2003-2004

<i>Shigella</i> type	Frequency	%
<i>S. Flexneri</i>	102	69.4
<i>S. Dysenteriae</i>	32	21.8
<i>S. Boydii</i>	11	7.5
<i>S. Sonnei</i>	2	1.3
Total	147	100.0

nalidixic acid (1.3%) and there was no resistance to ciprofloxacin and ceftriaxone (Table 2). The frequency distribution of these resistant strains was as follows: *S. flexneri* 71%, *S. dysenteriae* 18.4%, *S. boydii* 10.5%. *S. sonnei* resistance against these antibiotics was negligible.

DISCUSSION

The frequency of different species of *Shigella* varies in different countries. The most abundant species of *Shigella* in present study was *S. flexneri* which is similar to studies in Japan (12, 13), Ethiopia (14), India (15, 16), Senegal (17), Spain (18), Pakistan (19) and Kuwait (20). On the other hand, the most prevalent *Shigella* species in Australia (21) Poland (22), Turkey (10) and Saudi Arabia (23) have been *S. sonnei*. In our study, the least isolation was for *S. sonnei* (1.3%) which is similar to Ethiopia (14).

Results of studies in Poland (24) and Australia (21) have shown variations in prevalence of different species of *Shigella* in different parts of a single country (21, 24). In Lekehno, India, *S. sonnei* (4.6%) was the least reported species whilst in another city of India, Calcutta, *S. boydii* (9%) was reported as the least common species (15). In the latter study, most abundant species was *S. flexneri* whilst in former study *S. dysenteriae* was the most abundant species.

Table 2. The frequency of resistance pattern of *Shigella spp.* isolated from diarrheic patients in Zahedan in 2003-2004

Antibiotic	Frequency	%
Ampicillin	146	99.3
Trimethoprim-Sulfamethoxazole	84	57.1
Chloramphenicol	76	52
Nalidixic acid	19	1.3
Ciprofloxacin	0	0
Ceftriaxone	0	0

In the present study highest rate of resistance was observed against ampicillin (99.3%). This is similar to results of a study in Senegal in which all the isolated bacteria showed resistance to this antibiotic (17). Resistance rate to ampicillin in Kuwait, Turkey, Pakistan, Ethiopia and India has been reported as 54%, 72.9%, 55.5%, 70.1% and 69%, respectively (20, 10, 19, 14, 15). It is clear that application of this antibiotic is not appropriate. The result of a study in Fiji Island also emphasizes this finding (24).

After ampicillin, the highest resistance rate in our study was against trimethoprim-sulfamethoxazole (58.1%). In Ethiopia 5.3%, in Kuwait 56%, in Turkey 70%, and in Pakistan 87.75% of *Shigella* strains were resistant to trimethoprim-sulfamethoxazole (14, 20, 10, 19). Resistance rate has been reported to be more than 90% in India, and the effectiveness of this antibiotic in the treatment of shigellosis is reported to be reducing (15). In Poland only two *Shigella* species have reported as sensitive to this antibiotic (22). Therefore, it is suggested that application of trimethoprim-sulfamethoxazole must be confirmed by antibiogram. It seems that resistance to this antibiotic is correlated with the isolated species because the frequency of the resistance in *S. flexneri* has been about 50% whereas it has been more than 65% in *S. dysenteriae* and *S. boydii*. These differences emphasize the necessity to determine the species before clinical usage of antibiotics.

The least resistance in our study was against nalidixic acid (1.3%). In Senegal (17), India (25) and Poland (21), no resistance reported against this antibiotic whereas in Saudi Arabia 46.1% (23), Pakistan 39% (19), Vietnam 3.5% (26), Ethiopia 6.5% (14), Calcutta 29% (15) and southern India 94% (25) of the isolated bacteria were resistant to this antibiotic. In Japan resistance to this antibiotic has increased from 13.3% to 50% within a period of 7 years (12, 13). In Japan, more than half of the resistant strains are isolated from traveler diarrhea (27).

In our study, no resistance observed against ciprofloxacin and ceftriaxone. In similar studies in Vietnam and Kuwait there were also no resistance to ciprofloxacin (26, 20), and in Fiji Island these two antibiotics were reported effective for the treatment

of Shigellosis, although the treatment cost were reported more than the other applied antibiotics (24). In Calcutta, 4% of the isolated *Shigella* strains had moderate sensitivity to ciprofloxacin, indicating appearance of antibiotic resistant strains in India. It seems that *S. sonnei* isolation is increasing and its resistance to antibiotics such as ciprofloxacin and nalidixic acid is notable in India (15).

One of the problems in treatment is the appearance of multiple drug resistant strains. In the present study, 25.8% of studied strains had resistant to ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol. High resistance was observed to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole in Ethiopia (14). The comparison between the results of the present study with Ethiopian study shows that the frequency of the resistance, especially against ampicillin, trimethoprim-sulfamethoxazole and chloramphenicol, has been increased.

In the light of findings of our study, we strongly recommend that antibiotics should not be used without laboratory testing (antibiogram). More research is needed in this area and other antibiotics must be evaluated.

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Conflict of interests

We have no competing interests.

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