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Prevalence and Multiple Drug Resistance of *Shigella sonnei* Isolated from Diarrheal Stool of Children

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ABSTRACT

***Background*:** Science the discovery of antibiotic, the incidence of antibiotic resistance has been inevitable. Although there has been many study in these area but problem still exists. The aim of this research was to study the serotyping and multiple antibiotic resistance patterns of *Shigella sonnei* isolated from diarrheal stool of children.

***Methods*:** The stool samples of children from zero to fourteen years of age admitted at Children Medical Center in Tehran were tested over period of twelvemonth. Identification of isolates was carried out according to standard methods and the antibiotic susceptibility test was performed using Kirby Bauer disk method.

***Results*:** Of the 200 samples analyzed 6 (3%) were tested positive for *Shigella sonnei*. The antibiotic resistance patterns showed that all were résistance to tetracycline, streptomycin, clindamycin and cortimoxazol, and 66.7% of the samples had multiples resistance to above antibiotics.

***Conclusion*:** The results showed that multiple antibiotics resistance of *Shigella sonnei* is increasing, therefore awareness about the prevention by improved hygiene and proper medication are needed to reduce the burden of the preventable infectious diseases among young children.

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Introduction

Diarrheal disease is one of the most important causes of health threats in Iran and other developing countries especially in children (1). Diarrhea is one of leading causes of morbidity and mortality worldwide. Recent estimations suggested the number of deaths is close to 2.5 million (2). Among the pathogens causing diarrhea, *Shigella* species play an important role to cause bloody diarrhea. Identification of intestinal pathogens associated with diarrhea is a necessary step to prevent diarrheal diseases (3, 4). Antibiotic-resistant is the problem of everyday life in medical care system (5). The resistance to antibiotics is inevitable, which is a kind of defensive reaction of infectious agents, and it has been since the discovery of antibiotics (6). Although previous studies have shown that antibiotic resistance is increasing, but the patterns and extend of resistance are variable in different area due to bacterial diversity, therefore the evolution of antibiotic resistance pasterns in every region is of great importance (7). *Shigella flexeneri* especially type 2 was a dominant species in Iran for a Long time (8). But in recent years the situation has changed and now *Shigella sonnei* is the dominant strain especially in children (9, 10).Widespread outbreaks of shigellosis due to multiple drug resistance (MDR) strains with high mortality rates, have been documented in Asian countries (11-13). For these reasons we need constantly to identify the species of *Shigella* in children diarrheal in the country and to determine the antibiotic resistance pasterns. The aim of this research was to study the serotyping and evaluate the multiple antibiotic resistance patterns of *Shigella sonnei* isolated from diarrheal stool of children.

Material and method

This was a descriptive study conducted over period of one year from May 2013 to May 2014. A total of 200 stool samples of children with diarrhea admitted to the health center in Tehran were evaluated. Obtained from The inclusion criteria was the children age between zero and fourteen years and exclusion criteria was children over fourteen. Of age with diarrhea examined by culture. All the patients completed the epidemiological questionnaire information as recommended by the World Health Organization (14). Samples were taken by swab from the patient's stool and transferred to Cary Blair medium before sending to laboratory. The isolated were cultured on XLD, Hekton agar and incubated at 37C for 24 hrs. The colonies of small and round colorless (lactose negative) of the XLD and green colonies (lactose negative) of the Hekton agar were selected for the biochemical tests such as TSI, SIM, urea, Simon citrate, MR-VP, and lysine . By using standard tables (10) *Shigella* were identified and for the final approval agglutination testing using polyvalent antiserum Group D was used. Disk diffusion method was used for antibiotic susceptibility test. Ten antibiotic discs (Mast Co.UK) including ciprofloxacin, ampicillin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, streptomycin, nikarmylyn, cefotaxime, clindamycin, chloramphenicol were used. Results were analyzed according to CLSI (14).

Result

Of the 200 samples analyzed 6 (3%) were tested positive by biochemical and serological methods as *S. sonnei*. Four (67/66%) were from male and 2 (33/33%) from female. Three isolates were found in children aged between 1to 5 and 3 from 6 to 10 years old, respectively. There were no cases of *Shigella* isolate in11 to 14 years old children. The serological tests of isolates with *Shigella* antiserum were confirmed as *S. Sonnei* Group D. The results of antibiotic susceptibility test are shown in table 1 which indicates that all the *S. sonnei* isolates were resistance to tetracycline, cotrimoxazol, clindamycin, ticarcilin, streptomycin, while all were sensitive to ciprofloxacin, chloramphenicol, cefotaxin.

Table 1. Antimicrobial sensitivity of *Shigella sonnei* isolates (n=6).

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Tetracycline	0	0	6 (100)
Cotrimoxazol	0	0	6 (100)
Clindamycin	0	0	6 (100)
Ticarcilin	0	0	6 (100)
Streptomycin,	0	0	6 (100)
Ampicillin	3(50)	o	3(50)
Gentamaicin	3(50)	1 (16)	2 (33)
Ciprofloxacin	6(100)	0	0
Chloramphenicol	6(100)	0	0
Cefotaxin	6(100)	0	0

Table 2. The results of MDR of *Shigella sonnei* AMP: Ampicillin, TC: Ticarcilin, CTX: Cefotaxim, C: Chlormphenicol, GN: Gentamaicin, CP: Ciprofloxacin, CD: Cindamaicine, NA: Nalidixic acid, STR: Streptomycin, SXT: Co-trimoxsazole, TE: Tetracycline.

NO.	Antimicrobial resistance profile	Percent of isolate	MDR
1	TE/SXT/STR/ TC/CD	66/66	+
2	TE/SXT/STR/AMP	66/16	+
3	STR/AMP/CTX/TC/GN	66/16	+
4	TE/SXT/AMP/TC	66/16	+
5	CD/TC/ GN	66/16	+

The results of MD Rare shown in table 2 with resistance to three or more to antibiotics. It should be noted that (MDR) reported positive when three or more to, have shown resistance.

Discussion

Gastrointestinal infection with clinical sign of diarrhea causing by many different species of *S. sonnei* along with increased antibiotic resistance are problem in the world, especially in the third world countries (3, 7).The predominance of *S. sonnei* had also been reported elsewhere (14-16). In this study, 6 *S. sonnei* strains were isolated from children with acute diarrhea referring to Tehran Children Medical Center. Most patients were up to 10 years of age, this is in agreement with the previous study in elsewhere which could be due to lack of proper sanitary by young children (17). In other studies, male and female children were equally affected which is also in agreement with the previous study (18). Additionally, the rate of diarrhea was higher in the male than in female children as reported in many previous studies (19-22). Epidemiology studies and estimation of prevalence of diarrheal species can be a useful tool to identifying the

sources of contamination, amount of frequency, species dispersal and serotype in the country which could help us to control and prevent infections (23, 24). Our antibiotic susceptibility results showed that maximum sensitivity occurred with chloramphenicol, ciprofloxacin and cefotaxin, respectively, which was similar to other studies (23-26). A study carried out in USA showed that none of tested *S. sonnei* were resistance to ciprofloxacin or cefotaxin and only 1% were resistance to nalidixic acid (27). In our study all the tested *Shigella* were sensitive to ciprofloxacin. A study conducted in Iran has shown that level of resistance to ciprofloxacin was zero and to gentamycin was 12.5%, respectively (28).

Similarly, in our study the level of resistance to ciprofloxacin was zero but 33.3% were to gentamycin, which was relatively higher. Additionally, it has been reported in Iran that all the tested *Shigella* were sensitive to ciprofloxacin and ceftoaxin (29), similar to our study. However, they reported that81.3% of *Shigella* were resistance to tetracyclin, whereas in our study 100% were resistance to gentamycin (29). This is alarming and shows that the level of resistance is increasing. It is also noteworthy that none of the *Shigella* strains were resistant to ciprofloxacin, chlormphenicol and cefotaxin.

Conclusion

As a conclusion, antibiotic therapy using ciprofloxacin has shown advantages in the treatment of shigellosis, when reaching high concentration in serum and feces. In conclusion, this study revealed that there is a consistent increase in incidence of multiple drug resistance in *S. sonnei* strains isolates in Tehran, Iran.

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Conflict of interest

None declared conflicts of interest.

Financial disclosure

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