Original Article

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The Recent Trends of Shigellosis: A JIPMER Perspective

Microbiology Section

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ABSTRACT

Background: The multi-drug resistant *Shigella* has posed a therapeutic challenge in most parts of the world. In the last few years, there has been a tremendous change in the anti-microbial susceptibility profile of this organism.

Aim: This present study was carried out to determine the current anti-microbial susceptibility pattern of the members of the genus, *Shigella* in our region.

Materials and Methods: 2658 stool samples from patients with diarrhoea were received between 2008 and 2010. The disc diffusion testing was performed by the Kirby-Bauer method and the minimum inhibitory concentrations (MICs) of ciprofloxacin and ceftriaxone were obtained by the agar dilution method and the E-test. The double disk synergy test was used to confirm the status of the extended beta-lactamase producers.

Results: 74 (2.78%) *Shigella* spp were isolated, out of which *S.flexneri* was 90.54%, *S.dysenteriae* was 2.70%, *S.boydii* was 1.35% and *S.sonnei* was 5.40%. 43 (58.108%) strains were isolated from children of 0 to ≤5 years, 13(17.56%) were isolated from children who were >5 years but ≤ 15 years of age and the rest of the 18 (24.32%) were isolated from adult patients. 79% of the strains were resistant to ampicillin, followed by 51% which were resistant to nalidixic acid, followed by 50% which were resistant to ciprofloxacin (the MIC of ciprofloxacin was 16µg/ml), and 39.4% which were resistant to furoxone and chloramphenicol respectively. 2 (3%) strains of *S.flexneri* were found to be resistant to ceftriaxone, which had MICs of > 256µg/ml. The ceftriaxone resistant *S. flexneri* isolates were confirmed to be extended spectrum beta-lactamase producers by the double disk synergy test.

Conclusion: The continuous assessment of the anti-microbial susceptibility patterns and the periodic reporting in this context is important.

Key Words: Shigellosis, Antimicrobial resistance, *Shigella*, Ciprofloxacin, Ceftriaxone, Minimum inhibitory concentration

INTRODUCTION

The *Shigella* species cause an invasive gastroenteritis and are also causes of traveller's diarrhoea. In the travellers who go to the developing countries, diarrhoea is the most frequent symptom which is seen, when they are exposed to this infectious agent. Shigellosis is very common in children and it is one of the leading causes of morbidity and mortality in this group in the developing countries. In the endemic areas, the children who are less than 5 years of age have the highest rate of incidence. After the age of 5 years, the incidence declines, thus suggesting that a protective immunity develops after the exposure and that it is serotype specific [1,2].

Any member of the genus, *Shigella,* namely *S. dysenteriae, S. flexneri*, *S. sonnei* and *S.boydii,* can produce Shigellosis. Among the pathogenic Shigella subgroups, S. flexneri is the most commonly detected strain in the Asian countries [2].

Though the control of the severe disease, shortening the duration of the fever, diarrhoea and toxaemia, in reducing the risk of lethal complications, reducing the excretion of the pathogen in the stools significantly and reducing the spread of the infection warrants the appropriate chemotherapy, the unfortunate increase in the anti-microbial resistance across the globe has been an area for concern. Conventionally, the fluoroquinolones have been the mainstay of the treatment for Shigellosis. With the emergence of fluoroquinolone resistance among the Shigellae [2-7], the choice of the antibiotics has been reduced further.

The present study reflects upon the changing antibiotic resistance pattern in the *Shigella* spp. in our region.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology, JIPMER, Puducherry. A total of 2658 stool samples from patients with diarrhoea were received between 2008 and 2010. The stool specimens which were received were inoculated onto Mac Conkey's agar, deoxycholate citrate agar media, xylose lysine deoxycholate agar media and selenite-F enrichment broth, and these media were incubated at 37ºC overnight. Subcultures from the selenite-F broth was done after 18 hours onto Mac Conkey's agar, deoxycholate citrate agar media and xylose lysine deoxycholate agar media [10]. The *Shigella* strains were identified, based on their cultural characteristics and various biochemical tests [10] and they were confirmed by doing the slide agglutination test by using the specific antisera (Denka-Seiken, Tokyo, Japan). Disc diffusion testing was performed by using the Kirby Bauer method as per the Clinical Laboratory Standards Institute guidelines [11] against ampicillin (A)10µg, ceftriaxone (Ci)30µg, ciprofloxacin (Cf)5µg, nalidixic acid (Na)30µg, furoxone (Fx)300µg, chloramphenicol(C) 30µg and cotrimoxazole(Co) 25µg.

The Minimum Inhibitory Concentrations (MICs) of ceftriaxone and ciprofloxacin were determined by the agar dilution method and the E-test. For the agar dilution method, the ceftriaxone-Sodium

salt (Himedia, Mumbai, India) and the ciprofloxacin hydrochloride powder (Himedia, Mumbai, India) were dissolved respectively in sterile distilled water as was described by the manufacturer. The drugs, after their reconstitution, were stored at 4ºC and were used within 2 days of their reconstitution. Different dilutions of the antibiotics were used as per the recommendations [12,13]. ATCC *Escherichia coli* 25922 was inoculated on each plate as the growth control. The E-test was performed as per the manufacturers' instructions (Biomeriuex, India). The double disk synergy test was carried out on the ceftriaxone resistant isolates [13].

RESULTS

A total of 74 (2.78%) *Shigella* spp. were identified from the 2658 stool samples which were received in the Stool Section, Department of Microbiology, JIPMER, Puducherry, in the time period from January 2008 to December 2010 [Table/Fig-1]. These were isolated from patients whose ages ranged from 0 to 45 years.

Of these, 43 (58.108%) strains were isolated from children of 0 to ≤5 years, 13(17.56%) were isolated from children who were >5 years but ≤ 15 years of age and the rest of the 18 (24.32%) were isolated from adult patients [Table/Fig-2]. Only *S.flexneri* could be isolated from patients who were >15 years of age. The rest of the strains were found to be associated with the paediatric age group. The predominant manifestation which was common to all the strains was dysentery, except *S.boydii*, of which only one strain was isolated.

Out of the 74 isolates, 35 (50%) strains were resistant to ciprofloxacin, with an MIC of 16µg/ml. 2 (3%) strains were found to be resistant to ceftriaxone, with MICs of > 256µg/ml. These 2 ceftriaxone resistant isolates were identified as S. *flexneri*: one of these strains was isolated from a child who was <5 years of age and the other was isolated from an adult of 18 years of age. These 2 strains of *S. flexneri* were further confirmed to be ESBL producers phenotypically by the Double Disk Synergy Test by using a ceftazidime (Ca) 30µg and ceftazidime 30µg plus clavulanic acid 10µg (CaC) disk (Himedia, Mumbai, India).

DISCUSSION

Shigella has the capacity to invade the colonic epithelium causing micro-ulcers and crypt abscesses which lead to the appearance of blood and leucocytes in the stool. In the present study, the predominant outcome of the infection with any of the strains was dysentery, followed by acute gastroenteritis [Table/Fig-1]. This was in contrast to the clinical presentation in Peru [5], where acute gastroenteritis (AGE) was the commonest manifestation of Shigellosis, which was noted in 79.8% of the cases. 73.4% cases had fever associated with them, 30.8% had dysentery and 3.2% were asymptomatic. In the present study, AGE was observed in 28.37% cases, while dysentery was the commonest manifestation in 62.16% cases, chronic diarrhoea was noted in 5.45% cases and 4.05% cases showed Shigella encephalopathy. It was noted that only *S.flexneri* could be isolated from the paediatric as well as the adult age groups, whereas all the other strains remained confined to the paediatric age group. The manifestation of *S.flexneri* was more variable as compared to the other strains. The infection with any of the members of the genus, *Shigella* can result in persistent and chronic diarrhoea, especially in children with a protein energy malnutrition. We had 4 such children with chronic diarrhoea, from whom *S.flexneri* was isolated. Shigella encephalopathy was observed in three cases of infection with *S.flexneri* (Tablel). Shigella encephalopathy is a neurologic manifestation which arises due to metabolic derangements such as electrolyte imbalances or hypoglycaemia and it is mostly associated with *S.flexneri*. Clinically, altered consciousness with a bizarre posturing and unresponsiveness was noted [17].

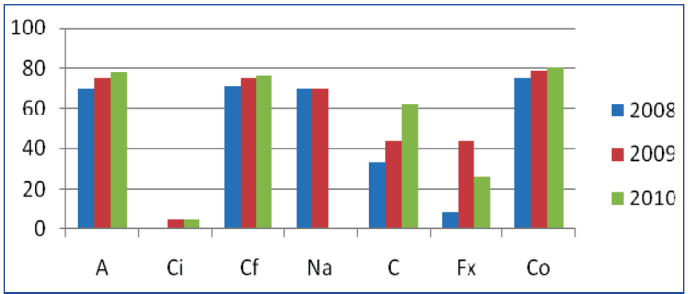
Although, the cyclical changes in the serotypes is a known fact, the factors which govern this phenomenon are exactly not known. The most common (90.54%) circulating serotype which was determined

Shigella spp.	Total Number (n=74)	Number of isolates in different age groups			Clinical manifestations associated	Antimicrobial resistance pattern in 2008	Antimicrobial resistance pattern in 2009	Antimicrobial resistance pattern in 2010
		0 to ≤5 years	≤15 years	≥15 years				
S. dysenteriae	2 (2.70%)	2 (100%)	0	0	Dysentery=1 AGE=1	A=Co	A=Co=Na=Cf=Fx=C	Nil
S. flexneri	67 (90.54%)	37 (55.22%)	12 (17.91%)	18 (26.86%)	Dysentery=42 AGE=18 Chronic diarrhoea=4 Shigella encephalopathy=3	A=Co>Na>Cf>C>Fx	A=Co=Na=Cf>C>Fx	A=Co>Cf>Fx=C>Ci Na was not tested
S. boydii	1 (1.35%)	1(100%)	0	0	AGE=1	A	A=Na=C=Fx=Co	Nil
S. sonnei	4 (5.40%)	3(75%)	1(25)	0	Dysentery=3 AGE=1	Co>A=Na=C>Cf=Fx	Nil	A=Cf=Fx=Co

[Table/Fig-1]: The distribution of the *Shigella* spp isolated from children and adults
Abbreviations used: A-Ampicillin, Na-Nalidixic acid, Cf-Ciprofloxacin, C-Chloramphenicol, Ci-Ceftriaxone, Fx-Furazolidone, AGE-Acute gastroenteritis.

Isolates from different places	Peru (2008) ref 5	Kolkata (2003) ref 3	Bangalore (2009) ref 4	PGIMER (2005) ref6	JIPMER (present study)
S.dysenteriae	2.4%	9.37%	3.7%	23.4%	2.70%
S.flexneri	67.1%	60.4%	64.9%	61.7%	90.54%
S.boydii	11.4%	6.4%	8.2%	7.45%	1.35%
S.sonnei	11.8%	23.8%	21.6%	7.45%	5.40%
Total	7.2%	-	4.6%	-	2.78%

[Table/Fig-2]: Comparison of isolation of Shigella spp.from different places



[Table/Fig-3]: Resistance pattern in *S.flexneri* over the years. Y–axis: percentage of strains resistant, X-axis: antibiotics tested; A-ampicillin, Ci-ceftriaxone, Cf-ciprofloxacin, Fx-furoxone, Co-cotrimoxazole

in our region was *S. flexneri,* followed by *S.sonnei, S.dysenteriae and S.boydii*. This trend of the strain variation pattern was probably similar to that of Kolkata [Table/Fig-2].

The commonest age group which was involved was clearly the children of < 5 years of age with 43 (58.108%) strains of *Shigella* being isolated from their stool samples [Table/Fig-2]. Children were more often involved than the adults (78.72% vs 21.27%). This finding was similar to that of a study which was done in Peru [5] where children were involved more than adults (80% vs 20%). It also showed similarity with a report from Chandigarh [18] in which children were involved more commonly (61.3%) than adults (29%) and it was in contrast to an earlier report from Bangalore [4], where children were less involved than adults (41% vs 58.2%).

The choice of the antibiotics for treating any infectious disease in children rests on a number of factors, especially the route of administration; among which the oral route is preferred. Unfortunately, the *Shigella* strains which were isolated from children in our centre were resistant to ampicillin and cotrimoxazole, in nearly >70% of all the cases, thus limiting the choice of the orally administrable antibiotics. A progressive trend of the resistance pattern was observed, with nearly >90% of the strains being resistant to more than two antibiotics by the turn of 2010. The most dramatic and alarming increase in the drug resistance was noted in *S.flexneri*. The strains (~80%) of *S.flexneri* which were isolated in 2010 were resistant to more than two groups of antibiotics as compared to those which were isolated in 2009 (~75%) and 2008 (~70%) respectively [Table/Fig-3]. Ciprofloxacin is very easily available and it is used most commonly, which has contributed to the resistance to this antibiotic. In the present study, the MIC values which were obtained were similar to that from earlier studies which were done in India [18]. More so, injectables like the third generation cephalosporins have not been spared. The high MIC value of ceftriaxone which was observed in some of our strains was an alarming finding. The emerging pattern of resistance in our centre was ampicillin>cotrimoxazole>nalidixicacid>ciprofloxacin>furoxone=chloramphenicol>> ceftriaxone, in the order of the decreasing resistance.

Ciprofloxacin has been recommended by the WHO [1] as the drug of choice for all the patients with Shigellosis, irrespective of their ages. Other alternatives to ciprofloxacin include ceftriaxone and azithromycin. The problems with ceftriaxone is that it is an injectable drug. Azithromycin is has limited therapeutic benefit as organisms easily develop resistance to it and paucity of data relating to its efficacy. In cases of strains which are ESBL producers,beta lactam with a beta lactamse inhibitor can also be tried [15].

The emergence of the ciprofloxacin resistance [18] has been widespread and this has shifted the focus to other alternatives, namely the cephalosporins. Unfortunately, the recent reports of cephalosporin resistance [14-16] has now really limited the options for the therapy.

The extended spectrum beta-lactamases (ESBLs) are on the rise both in terms of frequency of occurrence as well as the type of the class of the enzyme. ESBLs have been noted in *Shigella* as well. Molecular evidences have confirmed that the ESBL genes are plasmid borne. The *Shigella* species usually harbour a heterogeneous population of plasmids which range in numbers from 2 to as many as 10 [16]. The ability of these organisms to acquire the resistance genes which are located on the mobile elements, the continuous selective pressure which results from the high levels of the antibiotic consumption and the presence of the poor quality unlicensed antibacterial agents in some areas, enhances the likelihood of the development of resistance [2,7-9,13,14]. The molecular characterization of the strains is underway, which will throw more light on the details of the drug resistance.

The emerging drug resistance pattern has created a very dismal picture, with limited therapeutic options being available to combat it. The emergence of such strains can become a serious threat to the public health, as their spread among a population in which diarrhoeal disease is one of the major causes of childhood morbidity and mortality, calls for a greater attention to the appropriate use of the antibiotics, the establishment of hygienic measures to prevent or to decrease the transmission of this disease, and the development of new effective drugs that can be safely used in children. There is an impending need of updating the guidelines for the treatment of Shigellosis in the developing countries. In this context, the continuous assessment of the anti-microbial susceptibility patterns and stringent methods like strict adherence to the anti-microbial policies may place a check on this growing menace.

KEY MESSAGES

This study reflected and highlighted the increasing anti-microbial resistance in the *Shigella* strains which were isolated from our patients. Also, this brought into consideration, the emergence of the resistance in a number of anti-microbial agents, which included the third generation cephalosporins. This data may have a considerable impact on the treatment of Shigellosis and it highlights the role of the continuing surveillance for the drug resistance.

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