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Research Article

SPECIES DIVERSITY AND CHANGING PATTERNS OF ANTIMICROBIAL SUSCEPTIBILITY OF SHIGELLA SEROTYPES ISOLATED FROM STOOL SAMPLES IN A TERTIARY HEALTH CARE HOSPITAL OF PUNJAB, NORTH INDIA, A 5 YEAR STUDY

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ABSTRACT
Shigella is an important cause of invasive dysentery in children and others. The present study was done to study the prevalence, any seasonal variation, distribution among children and adults, and antimicrobial susceptibility pattern of Shigella isolates in stools. A retrospective study was conducted over a period of 5 year from 1 January 2009 to 31th December 2013 in the department of microbiology, in a tertiary care hospital in Punjab. Stool specimens from diarrhea/dysentery cases in different age-groups were processed. The specimens were processed by standard microbiological techniques. The suspected colonies of Shigella were identified by standard biochemical tests and serotyping was done by group specific antisera. The susceptibility of all the isolated Shigella species to different antibiotics were done by Kirby-Bauer's disk diffusion technique as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Shigella species were isolated from 146 (2.42%) of the total 6025 stool samples received during the study period. Among these, *S.flexneri* was the predominant species accounting for 67.1% of isolates, followed by *S.sonnei* 25.3%, *S.boydii* 5.4% and S. *dysenteriae* in 2.1% of the total number of isolates. Of these, 53% were from children from 0-18 years and the rest 47% were from adults. Over 70% of Shigella isolates were resistant to two or more drugs including ampicillin, and co-trimoxazole and approximately, 3% *Shigella* isolates were resistant to ceftriaxone, during the study period. Analysis of the antibiotic susceptibility data showed a gradual increase in resistance to ampicillin, fluctuating resistance to norfloxacin and persistent resistance to co-trimoxazole. No resistance was observed to cefoperazone/sulbactam, piperacillin/tazobactam, and imipenem during the study period. The results of the study revealed the high prevalence of shigellosis with *S. flexneri* as the predominant species. The result also suggests that ampicillin, co-trimoxazole and norfloxacin should not be used empirically as the first line drugs in the treatment of shigellosis. Periodic analysis and reporting of antibiotic susceptibility is an important measure to check for antibiotic resistance and form appropriate treatment protocols.

Keywords: *Antimicrobial Resistance, Dysentery/Diarrhea, India, Shigella, Shigellosis*

INTRODUCTION
Shigellosis is a public health problem; especially in developing countries where about 163 million people are affected annually, among which 69% of cases occur in children less than 5 years. (Wkly Epidemiol Rec 2005; Niyogi, 2005) Intestinal infection with Shigella species can be managed by rehydration therapy; however, treatment with antimicrobial agents has been proven effective in reducing intensity and duration of symptoms and also in preventing lethal complications (Peirano, 2006). Shigella sp. were susceptible to many antimicrobials initially, but they have developed resistance to them over a period of time. Antimicrobial susceptibility patterns of Shigella sp. vary among its serogroups and also between geographical areas periodically (Bhattacharya, 2012). A local knowledge of the distribution of Shigella serogroups, with their changing drug resistance patterns to guide empiric antimicrobial therapy is imperative in controlling shigellosis. Hence, the present study was conducted to find the prevalence, any seasonal variation, distribution among children and adults, and drug resistance pattern of Shigella isolates in North India.

MATERIALS AND METHODS

The study was conducted in the Microbiology Department of Christian Medical College and Hospital, Ludhiana, a tertiary care hospital in Punjab. A retrospective analysis was conducted over a period of 5 year from 1 January 2009 to 31th December 2013. During this period, 6025 stool samples were collected from diarrheal/dysenteric patients admitted to the hospital. The patients included children, adults, and elderly people.

Fresh faeces of patients were collected and transported immediately for culture. A battery of culture media including MacConkey agar, Deoxycholate citrate agar (DCA), and Selenite-F (SF) enrichment broth were used for the isolation of organisms. All media were incubated at 37°C for 24 h. MacConkey and DCA plates were examined and subcultures from SF were done on DCA.

The nonlactose fermenting colonies were subjected to standard biochemical tests using Triple sugar iron agar, Mannitol motility medium, and Indole production. Organisms biochemically resembling Shigella was serologically confirmed by specific antisera (Seiken, Japan). The susceptibility of all the isolated Shigella to different antibiotics was determined by Kirby-Bauer's disk diffusion technique as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics used were Ampicillin (10µg), Co-trimoxazole (25µg), Chloramphenicol (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Norfloxacin (10µg), Ceftriaxone (30µg), Cefoperazone/Sulbactam (75/30µg), Piperacillin/Tazobactam (100/10µg), and Imipenem (30µg).

RESULTS

Of the 6025 stool samples screened, Shigella strains were identified in 146(2.42%) samples. Shigella sp. were isolated from patients with ages ranging between 0 and 70 years. Of these, 53% were from children from 0-18 years and the rest 47% were from adults. The clinical course was found to be more severe in children with superadded malnutrition and the peak being during the monsoons, which declined remarkably with change of season. Males outnumbered the females in all the age-groups. *S. flexneri* strains were identified in 98 (67.1%) Shigella positive cultures, while *S.sonnei* accounted for 37 (25.3%). *S.boydii* was isolated in 8 (5.4%) and *S. dysenteriae* in 3 (2.1%) of the total number of isolates. *S. flexneri* has been predominant isolate during the period of the study. However the percentage of S.sonnei increased in 2012 and 2013 from an average of 17% to 33% (Table1).

Table 1: Prevalence of Shigella spp. from 2009 to 2013 in Ludhiana Year wise prevalence of Shigella spp

Year	*S. flexneri*	*S. sonnei*	*S. boydii*	*S. dysenteriae*
2009 (n=24)	20 (83.3)	2(8.3)	2(8.3)	0(0)
2010 (n=22)	12(54.5)	7(31.8)	2(9)	1(4.5)
2011 (n=28)	21(75)	5(17.8)	1(3.6)	1(3.6)
2012 (n=39)	24((61.5)	13(33.3)	1(2.5)	1(2.5)
2013 (n=33)	21(63.6)	10(30.3)	2(6.0)	0(0)
Total (n=146)	98(67.1)	37(25.3)	8(5.4)	3(2.1)

Values in parentheses represent percentage

The prevalence of resistance pattern of the isolated Shigella species during the study period 2009 to 2013 is as shown in the (Table2). Over 70% of Shigella isolates were resistant to two or more drugs including ampicillin and co-trimoxazole. Resistance rate of ampicillin was 65.7%, co-trimoxazole 87%, chloramphenicol 6.1%, norfloxacin 41.7%, gentamicin 2.7% and ciprofloxacin 21.2%. Approximately, 3% *Shigella* isolates were resistant to ceftriaxone, during the study period. No resistance was observed to cefoperazone/sulbactam, piperacillin/tazobactam, and imipenem during the study period. Considering prevalence of resistance pattern in different serogroups, *S. flexneri* showed highest resistance to co-trimoxazole (88%) and ampicillin (74%), *S.sonnei* to co-trimoxazole (86%) and ampicillin (43%),

S.*boydi*i to ampicillin (75%) and co-trimoxazole (50%) and S. *dysenteriae* to co-trimoxazole (100%) and ciprofloxacin (100%) (Table 2).Year wise resistance pattern in Shigella spp. is shown in figure.

Table 2: Prevalence of resistance pattern of Shigella spp. To different antibiotics

Serogroup	Amp	Cotri	Chlo	Norf	Gent	Cipr	CTR
S.flexneri (n=98)	73(74.4)	87(88.7)	8(8.1)	44(44.8)	3(3)	25(25.5)	3(3)
S.sonnei (n=37)	16(43.2)	32(86.4)	1(2.7%)	13(35.1)	1(2.7)	0(0)	0(0)
S.boydii (n=8)	6(75)	4(50)	0(0)	4(50)	0(0)	2(25)	1(12.5)
S.dysenteriae (n=3)	1(33.3)	3(100)	0(0)	0(0)	0(0)	3(100)	0(0)
Total (n=146)	96(65.7)	126(87)	9(6.1)	61(41.7)	4(2.7)	30(21.2)	4(2.7)

Values in parentheses represent percentage
Amp-Ampicillin, Co-trim-Cotrimoxazole, Chloro-Chloramphenicol, Norf-Norfloxacin, Genta-Gentamicin, Cipro-Ciprofloxacin, CTR-Ceftriaxone,

DISCUSSION

Shigellosis is endemic in our region as evidenced by the continued isolation of Shigella over the 5 year period. In this study, we isolated 146(2.42%), Shigella strains from diarrheal/dysenteric stools of 6025 patients. The percentage over 5 years varied between 2.5% to 4.5%. This rate is similar to studies done in Nepal and Israel and other parts of India, but is considerably lower than rates seen in other developing countries (Ashkenazi, 1995; Bhattacharya, 2005; Pazhani, 2005; Taneja, 2003; Legros, 1998; Mache, 2001). This difference could be due to underreporting of cases of shigellosis, improved public health measures, and continuing efforts by the health authorities to improve sanitation. The pattern of shigellosis indicates that *S. flexneri* was the most predominant and most active serogroup in Ludhiana during the years followed by *S. sonnei, S. boydii and S. dysenteriae*. This is comparable to the serogroup distribution seen in India and other developing countries. (Mamatha, 2007; Opintan, 2007; Ergönül, 2004) It is well-known that *S flexneri* is the predominant serogroup in most of the developing countries, while *S. sonnei* is the predominant species in developed countries. (Wilson, 2006; Hossain, 1990) The number of faecal samples submitted to the laboratory were more or less similar in all the 5 years but in 2012 more Shigella were isolated (4.5%).

The pattern of shigellosis indicates that *S. flexneri* was the most commonly isolated serogroup in Ludhiana during the years; however, in 2010 *S. sonnei* was isolated from 32% of cases reducing *S.flexneri* from near 84% to 55%. In 2011, *S.sonnei* had been reduced by 17% and isolation rate of *S.flexneri* increased from 55% to 75% (Table 1).This cyclic change agrees with the pattern observed in Puducheery and Kolkata where cyclical serogroups change had been observed over the years (Taneja, 2007; Dhodapkar, 2008).

Analysis by age showed that 80(53%) were from pediatric age group (0-18 years).The clinical course was found to be more severe in children with superadded malnutrition .This calls for an urgent measure to reduce the deaths in children due to Shigellosis. Therefore, public health strategy should ensure clean water supply, good sewage management and a clean environment.

Analysis of the antibiotic susceptibility data showed a gradual increase in resistance to ampicillin, fluctuating resistance to norfloxacin and persistent resistance to co-trimoxazole. Over 70% of Shigella isolates were resistant to two or more drugs including ampicillin, co-trimoxazole. which is similar to the resistance trend observed in other studies; (Mamatha, 2007; Opintan, 2007; Ergönül, 2004; Wilson, 2006; Hossain, 1990; Taneja, 2007) while there was least resistance to ceftriaxone. In our study, there is gradual increase in ampicillin resistance, 48% to 73% from 2009 to 2013. Co-trimoxazole resistance decreased from 95% in 2009 to 81% in 2013, but still high enough to discourage empiric use. Ampicillin and Co-trimoxazole were one of the commonly used drugs for the treatment of Shigellosis, but their indiscriminate use has resulted in development of resistant strains (Bennish, 992). Initially, resistance to

these two drugs were reported in certain strains of *S.sonnei* and *S.dysenteriae* in West Bengal during the 1990s, with similar resistance profile documented in isolates of *S. flexneri* in this region around the same time (Dutta, 1989; Pal, 1984). Resistance to co-trimoxazole is caused by a variant dihydrofolate reductase (DHFR) enzyme encoded by a plasmid-mediated gene (Navia, 1999). There is gradual decrease in fluoroquinolones resistance, 62% to 42% from 2009 to 2011 and then increase resistance up to 51% during the year 2012 and 2013. Resistance to fluoroquinolones among the Shigella isolates in this study was found to be high with high rates of resistance observed to norfloxacin than ciprofloxacin. A similar trend of increased fluoroquinolone resistance has been observed in India and other parts of the world, particularly in the Asian and African countries (Taneja, 2007; Gu, 2012). Flouroquinolones are recommended as the drug of choice for shigellosis by the World Health Organization. The emergence of fluoroquinolone resistant Shigella may be due to overuse of these drugs as they are empirically used for many infections like diarrhea and urinary tract infection. With the emergence of high resistance to fluoroquinolones, therapeutic options are limited. Third generation cephalosporins, pivmecillinam, and azithromycin are alternative drugs, which can be used for treatment of shigellosis. In our study, most of the isolates were susceptible to chloramphenicol, Ceftriaxone and furazolidine and no resistance to cefoperazone/sulbactum, piperacillin/tazobactam and imipenem was seen. This was similar to study by Mamatha *et al*., at Manipal (Mamatha, 2007).

Conclusions

The study shows the occurrence of drug resistant shigellosis. Antibiotics may not be necessary for mild cases and if required should be guided by local data. The continuous surveillance of multidrug strains is very important to know the changing antibiotic susceptibility pattern, as well as the cyclical change of the serogroup from time to time as the resistance pattern also changes with the change in the serogroup. Analysis and periodic reporting is important in proper therapy of shigellosis.

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