Original Article

Changing resistance pattern of *Shigella* isolates over a period of five years

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ABSTRACT

Background and objectives: Diarrheal diseases are an important cause of morbidity and mortality in children in developing countries. Among diarrheagenic agents, *Shigella* should be emphasized because of its prevalence and the severity of the associated disease. The present study was done to study the prevalence and antimicrobial susceptibility pattern of *Shigella* isolates in stool. **Materials and methods:** Stool samples were collected from cases of dysentery and diarrhea in the laboratory from Jan 2009 to Dec 2013. The specimens were processed and inoculated as per standard protocol. The susceptibility of *Shigella* serogroups to different antibiotics was done as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic susceptibility between different *Shigella* serogroups was compared and trends of drug resistance to various antimicrobial agents over a period of five years was seen. **Results:** Of a total of 6117 samples, *Shigella* serogroups were isolated in 74 (1.2%) samples. *S. flexneri* was the most common serogoup identified followed by *S. boydii* and *S. sonnei*. Yearwise isolation of *Shigella* serogroups was 1.58% in 2009 which decreased to 0.38% in 2013. All *Shigella* serogroups showed higher sensitivity to ciprofloxacin as compared to nalidixic acid, cotrimoxazole and ampicillin. **Interpretation and conclusion:** There is a significant increase in resistance to several commonly used antimicrobial agents. The rapid increase in ciprofloxacin resistance, especially in *S. flexneri*, is a major cause of concern. The results suggest reconsideration of the empiric use of these antimicrobial agents for the treatment of shigellosis.

Keywords: Antimicrobial resistance, diarrhea, *Shigella*

INTRODUCTION

Diarrheal diseases are an important cause of morbidity and mortality in developing countries. Of the bacterial causes of dysentery, *Shigella* serogroups are the major enteropathogens with outbreak potential and common development of antimicrobial resistance.[1] Among diarrheagenic agents, *Shigella* should be emphasized because of its prevalence and the severity of the associated disease, accounting for 140 million cases globally per year and 60,000 deaths annually of which 60% occur in children below 5 years of age.[2] Recent reports from certain Asian countries show incidence rates of shigellosis ranging between 3-13%.[3] Intestinal infection with *Shigella* serogroups 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.[4] Antimicrobial therapy is advocated for shigellosis to shorten the duration of illness. However, in Asia and Africa, antimicrobial resistance is an emerging problem among *Shigella* serogroups and treatment options are becoming limited globally.[5] *Shigella* sp. were susceptible to many antimicrobials initially, but 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.[6] A local knowledge of the distribution of *Shigella* serogroups,

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with their changing drug resistance patterns to guide empiric antimicrobial therapy is imperative in controlling shigellosis. Hence the present study was conducted to know the changing prevalence and antimicrobial susceptibility pattern of shigellosis over a period of five years.

MATERIALS AND METHODS

This was a retrospective study done over a period of 5 years. Stool samples were collected from cases of dysentery and diarrhea in all age groups from Jan 2009 to Dec 2013. The specimens were processed and inoculated on MacConkey's agar, deoxycholate citrate agar (DCA) and in selenite F enrichment broth. After 18-24 h, subculture was done on DCA. The plates were incubated aerobically at 37^0C overnight. Non-lactose fermenting colonies were identified on the basis of various biochemical tests and serologically *Shigella* was confirmed by specific antisera (Denka 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.[7] The antibiotics used were ampicillin (10μg), cotrimoxazole (1.25/23.75μg), ciprofloxacin (5 μg) and nalidixic acid (30μg). Antibiotic susceptibility between different *Shigella* serogroups was compared and trends of drug resistance to various antimicrobial agents over a period of five years was seen. Institutional Ethical Committee approval was obtained.

RESULTS

Of a total of 6117 stool samples received over a period of five years. *Shigella* serogroups were identified in 74 (1.2%) of them *S. flexneri* (93.2%) was the most common serogroup followed by *S. boydii* (4.05%) and *S. sonnei* (2.7%). Yearwise isolation of *Shigella* serogroups was 1.58% in 2009, 2.01% in 2010 which decreased to 1.6% in 2011, 0.61% in 2012 and 0.38% in 2013 (Table 1).

Table 1

Yearwise distribution of *Shigella* serogroups

Year	Total samples	*S. flexneri* (n= 69)	*S. sonnei* (n=2)	*S. boydii* (n=3)	Total (%) (74)
2009	1202	19	–	–	1.58%
2010	1140	21	–	2	2.01%
2011	1187	18	1	–	1.6%
2012	1294	6	1	1	0.61%
2013	1294	5	–	–	0.38%

Table 2

Percentage of *Shigella* serogroups resistant to antimicrobial agents

Antimicrobial agents	*S. flexneri* (n= 69)	*S. boydii* (n=3)	*S. sonnei* (n=2)
Ampicillin	46 (66.6%)	1(33.3%)	1(50%)
Cotrimoxazole	54 (78.3%)	2 (66.6%)	2 (100%)
Ciprofloxacin	18 (26.1%)	0 (0%)	0 (0%)
Nalidixic acid	65 (94.2%)	2 (66.6%)	2 (100%)

All *Shigella* serogroups showed higher sensitivity to ciprofloxacin as compared to nalidixic acid (93.2%), cotrimoxazole (78.3%) and ampicillin (64.8%) where high degree of resistance was observed (Fig.1) *S. sonnei* showed high resistance to nalidixic acid and cotrimoxazole whereas *S. flexneri* showed high resistance to ampicillin as compared to other serogroups (Table 2) Over the 5 year period, resistance to ampicillin increased from 42.4% in 2009 to 80% in 2013 and to ciprofloxacin from 15.8% to 40% (Fig. 2). Of the *Shigella* isolates, 81.5% were multidrug resistant, being commonly resistant to nalidixic acid and cotrimoxazole.

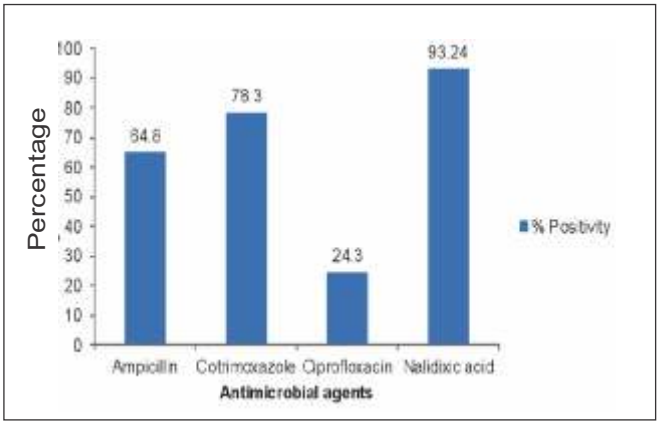

Fig.1: Antimicrobial resistance profile of *Shigella* isolates

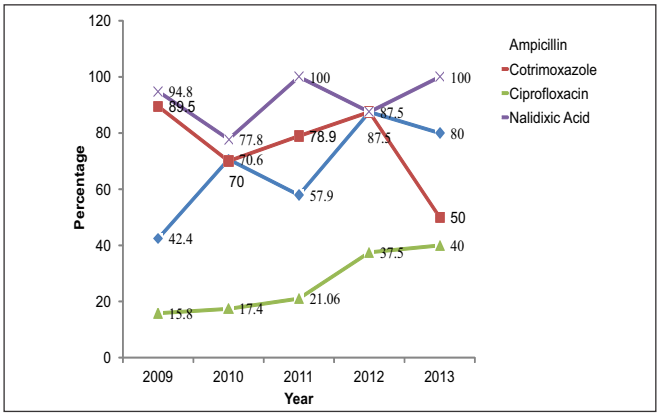

Fig.2: Percentage resistance of *Shigella* isolates to various antimicrobial agents (2009-2013)

DISCUSSION

Shigellosis still accounts for a significant proportion of morbidity and mortality, especially in developing countries.[8] In the present study, *Shigella* sp. were isolated from 1.2% stool samples. This rate is similar to studies done by Kumar *et al*[8] and Mandal,[9] whereas higher incidence of 6.47% was reported by Tejashree *et al*.[10] Rate of *Shigella* isolation has decreased from 2011 onwards. This difference could be due to improved public health measures, and continuing efforts by the health authorities to improve sanitation.

The pattern of shigellosis indicates that *S. flexneri* (93.2%) was the most commonly isolated serogroup. This is comparable to the serogroup distribution seen in India and other developing countries[9,11-12] where *S. flexneri* was the commonest whereas Rahbar *et al*[13] showed *S. sonnei* as the commonest *Shigella* isolate.

Antimicrobial therapy is recommended for shigellosis because it can shorten the severity and duration of illness, reduce shedding of the organism, and prevent secondary complication and death. However antimicrobial resistance occurred among *Shigella spp*, since the 1940s, when sulfonamide resistance was first recognized in Japan.[14] The present study showed high resistance to nalidixic acid (93.2%) followed by co-trimoxazole (78.3%) and ampicillin (64.8%). Similar resistance pattern to nalidixic acid (100%) and cotrimoxazole (76.9%) was observed by Patil *et al*.[15] Resistance pattern of ampicillin and cotrimoxazole observed by Kumar *et al*[8] was comparable to our study. In contrast lower resistance to nalidixic acid (51%) was observed by Mandal *et al*[9] and to cotrimoxazole in a study from Mysore.[10]

Fluoroquinolones are recommended as the drug of choice for shigellosis by the World Health Organization.[8] 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. The overall incidence of resistance to ciprofloxacin was 24.3%, which can be compared with other study done in Punjab showing a resistance of 13.3%.[8] However a very high resistance to ciprofloxacin has been reported from other parts of India.[9,11]

Over the five years, resistance to nalidixic acid was the maximum and least to ciprofloxacin. Resistance to ampicillin and cotrimoxazole was comparably the same in 2010 and 2012. A similar resistance pattern was reported by Tejashree *et al*[10] whereas higher resistance

to cotrimoxazole compared to ampicillin was reported by Kumar *et al.*[8] Of the *Shigella* isolates, 81.5% were multidrug resistant. The most common multidrug resistance pattern was to nalidixic acid and cotrimoxazole. Similar rate of multiple drug resistant *Shigella* was seen in other studies.[8,13]

In conclusion, the present study demonstrates that *S. flexneri* is the predominant serogroup. There is a significant increase in resistance to several commonly used antimicrobial agents. The rapid increase in ciprofloxacin resistance, especially in *S. flexneri*, is a major cause of concern. The results suggest reconsideration of the empiric use of these antimicrobial agents for the treatment of shigellosis.

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CONFLICT OF INTEREST: Nil

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