Species diversity and antimicrobial resistance of *Shigella* spp. isolated between 2001 and 2004 from hospitalized children with diarrhoea in Kolkata (Calcutta), India

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SUMMARY

The incidence, phenotypic characteristics and antimicrobial resistance patterns of 193 *Shigella* strains isolated from 2489 hospitalized children with acute diarrhoea were studied during January 2001 to August 2004. *S. flexneri* (60 %) was the most prevalent serogroup, followed by *S. sonnei* (23·8 %), *S. dysenteriae* (9·8 %) and *S. boydii* (5·7 %). Since 2002, *S. flexneri* 2a was the most dominant serotype. Almost all *S. flexneri* strains exhibited resistance to ampicillin, co-trimoxazole, tetracycline, nalidixic acid and fluoroquinolones. After a lapse of almost 14 years, *S. dysenteriae* type 1 strains reemerged for the first time during 2002 and these strains were resistant to more than two antibiotics (multidrug resistance), including fluoroquinolones. An upsurge of similar resistance patterns was also noted among *S. flexneri* type 2a since December 2003. Resistance to fluoroquinolone increased year on year among *S. dysenteriae* type 1 and *S. flexneri*, but not in *S. boydii* or *S. sonnei*. Monitoring of antimicrobial susceptibility through a surveillance programme is recommended to select appropriate antibiotics for the effective treatment of shigellosis in this region.

INTRODUCTION

Shigella spp. cause acute debilitating diarrhoeal disease in humans worldwide. It has been estimated that each year more than 163 million episodes of endemic shigellosis occur in developing countries accounting for 1·1 million deaths including 580 000 children [1, 2]. Person-to-person transmission accounts for most infections, which occur most commonly in children between the ages of 6 months and 10 years [3]. Four species of *Shigella* (*S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*) are responsible for the disease and each species is subdivided into serotypes with *S. dysenteriae*

comprising 12 serotypes, *S. flexneri* 6, *S. boydii* 18 and *S. sonnei* 1.

Over the past decades, *Shigella* spp. have become progressively resistant to the commonly used inexpensive antimicrobial compounds [4]. Moreover, changes in the incidence of *Shigella* spp. from time to time with altering patterns of antimicrobial susceptibilities make it difficult to recommend effective drugs for the treatment of shigellosis [5, 6]. In all acute diarrhoeal infections, the preferred and widely accepted treatment is rehydration, antimicrobial treatment, and nutritional therapy. Antimicrobial therapy for severe shigellosis in developing countries normally includes ampicillin, co-trimoxazole, nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin and pivmecillinam [7]. However, some *Shigella* spp. have developed resistance to most of the widely used fluoroquinolones

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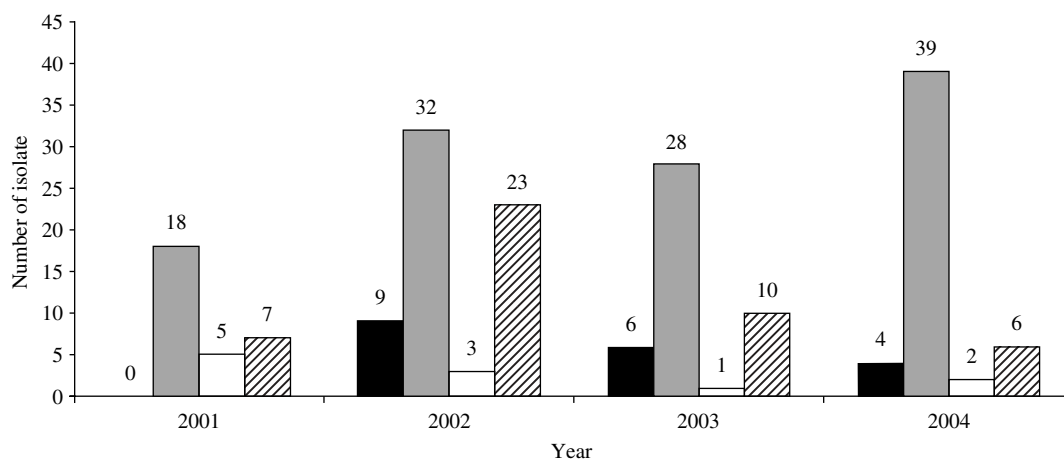(Email: niyogisk@hotmail.com)

Fig. 1. Incidence of different species of *Shigella* among hospitalized children with diarrhoea in Kolkata. ■, *S. dysenteriae*; ▨, *S. flexneri*; □, *S. boydii*; ▨, *S. sonnei*.

[8–10]. We report here, the incidence, phenotypic characteristics and antimicrobial resistance patterns of *Shigella* spp. isolated from hospitalized children with acute diarrhoea.

MATERIALS AND METHODS

Bacterial strains

During January 2001 to August 2004, a total of 2489 stool samples were collected from hospitalized children suffering from acute diarrhoea attending the Dr B. C. Roy Memorial Hospital for Children, Kolkata, India. Faecal samples were collected into sterile 'MacCartney' bottles or with rectal swabs in Cary–Blair transport medium and examined for common enteric pathogens within 2 h of collection, using standard microbiological and biochemical methods [11]. *Shigella* strains were confirmed serologically by slide agglutination using commercially purchased antisera (Denka Seiken Co. Ltd, Tokyo, Japan).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed by a disk diffusion method in accord with the National Committee of Clinical Laboratory Standards guidelines [12]. The following disks (Difco, Detroit, MI, USA.) were used, ampicillin (10 μg), co-trimoxazole (25 μg), tetracycline (30 μg) nalidixic acid (30 μg), ciprofloxacin (5 μg), norfloxacin (10 μg), and ofloxacin (5 μg). *Escherichia coli* ATCC 25922 strain was used for quality control in each batch of tests. The minimal inhibitory concentrations (MIC) of nalidixic

acid, ciprofloxacin, norfloxacin and ofloxacin were determined for selected quinolone-resistant strains by the E test (AB Biodisk, Solna, Sweden) following the manufacturer's instructions.

RESULTS

During this study, 193 (7·7%) *Shigella* strains were isolated from 2489 diarrhoeal patients. The distribution of the species were: 117 (60·6%) *S. flexneri*, 46 (23·8%) *S. sonnei*, 19 (9·8%) *S. dysenteriae* and 11 (5·7%) *S. boydii*. The year-wise incidence of the serotypes is shown in Figure 1. The incidence of *S. flexneri* was highest in 2002 and 2004 and *S. dysenteriae* type 1 was isolated for the first time in 2002, having been last recovered in 1988; two strains of serotype 2 of this species were isolated only during 2002. Except for type 12 (3 strains), there was no serotype predominance among a total of 11 *S. boydii* strains. Throughout the study period, *S. flexneri* was the most prevalent serogroup and *S. flexneri* serotype 2a was predominant during 2002–4 (Fig. 2), the frequency of the latter reached 14% during 2004. An increase in the incidence of serotypes 3a and 6 was also recorded during 2002.

During 2001, 50% of *Shigella* isolates were resistant to ampicillin, 96% to co-trimoxazole, 83% to tetracycline and 56% to nalidixic acid, but none of them was resistant to norfloxacin, ciprofloxacin or ofloxacin. Multidrug resistance was detected (13%) with ampicillin, co-trimoxazole and nalidixic acid/co-trimoxazole, and tetracycline and nalidixic acid, but 23% were resistant to the combination of ampicillin, co-trimoxazole, tetracycline and nalidixic acid. In 2002, ampicillin resistance was reduced to 32% from
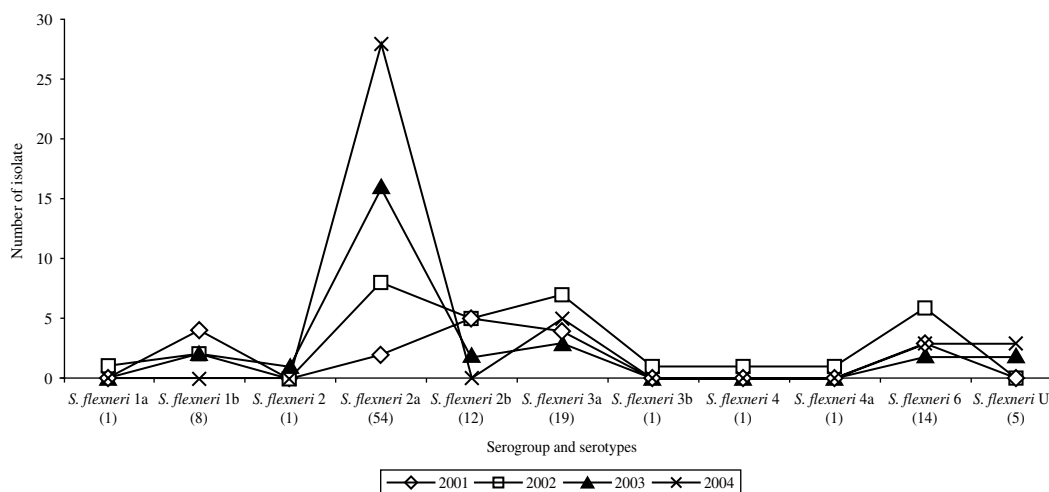
Fig. 2. Incidence of different serotypes of *S. flexneri* isolated between 2001 and 2004. –◇–, 2001; –□–, 2002; –▲–, 2003; –×–, 2004. Numbers in parentheses indicate number of strains. UT, Untypable.

50% in 2001. Similarly, co-trimoxazole resistance was also reduced to 83% from 93%, but tetracycline and nalidixic acid resistance increased by 6% over 2001. In addition, fluoroquinolone-resistant strains among *S. dysenteriae* and *S. flexneri* were identified during 2002 with increases in resistance year on year: 2002 (11%), 2003 (15%), and 2004 (25%). Antimicrobial resistance was higher among *S. dysenteriae* type 1 and *S. flexneri* than other serogroups (Table 1). *S. dysenteriae* type 1 strains were uniformly resistant to most of the commonly used antibiotics over the period of time and the MIC range for ciprofloxacin, norfloxacin, and ofloxacin during 2002 was 2–6, 6–24 and 8–16 μg/ml respectively. The other serotypes of *S. dysenteriae* showed uniform sensitivity to all antimicrobials tested.

Multidrug resistance was common among the *S. flexneri* strains irrespective of serotypes compared with *S. sonnei* and *S. boydii* (Table 2). In 2002, only one fluoroquinolone-resistant strain of *S. flexneri* serotype 3b was isolated, and its MICs for nalidixic acid, ciprofloxacin, norfloxacin and ofloxacin were >256, >200, 250, and >32 μg/ml respectively. However, from December 2003, few strains of *S. flexneri* serotype 2a were resistant to fluoroquinolone and the MIC of these strains were >256, 4–8, 10–16, and 12–16 μg/ml for nalidixic acid, ciprofloxacin, norfloxacin and ofloxacin respectively. *S. boydii* was the least isolated species during the study period and only reduced susceptibility to co-trimoxazole and tetracycline was observed. Since 2002, none of the *S. boydii* strains were resistant to quinolones. Among *S. sonnei* and *S. boydii* strains, fluoroquinolone resistance was not detected.

As shown in Table 2, most of the *S. dysenteriae* type 1 strains had the resistance profile Am Sxt Te Na Cip Nor Ofx. The same profile was also recorded among one *S. flexneri* 3b strain each during 2002–3 and eight strains of *S. flexneri* 2a in 2004. Three *S. flexneri* strains isolated during 2004 had the resistance profile of Sxt Te Na Cip Nor Ofx. Among *S. sonnei* strains, the most prevalent multidrug resistance profile was Sxt Te Na (Table 2).

DISCUSSION

The frequency of occurrence of *Shigella* spp. differs by country and in different populations within a country [13–16]. In this study, *S. flexneri* was the most common species, followed by *S. sonnei*, *S. dysenteriae* and *S. boydii*. This is in keeping with prevalence data in other developing countries [13–16]. In contrast, *S. sonnei* accounts for 64% of all *Shigella* spp. isolated in the United States, and 80% in European countries and Canada [17]. *S. dysenteriae* type 1 re-emerged during 2002 having been last isolated 14 years previously. A similar phenomenon was also recorded in Indonesia [18] and cyclic epidemic patterns of this serotype with substitution by *S. flexneri* and *S. sonnei*, have been observed since the turn of the century [19].

Studies from various parts of India have shown that *S. sonnei* was the second dominant species in Vellore, South India [20] and *S. boydii* in Chandigargh, North India [21]. In Kolkata, eastern India, *S. boydii* was the second most common species during 1995–6 and was replaced by *S. sonnei* in 1998 [5]. After the 1984 epidemic in eastern parts of India, *S. dysenteriae*

Table 1. *Percentage of antimicrobial resistant isolates of* Shigella *spp.*

Antimicrobial agents	S. dysenteriae				S. flexneri				S. boydii				S. sonnei			
	Year				Year				Year				Year			
	01	02	03	04	01	02	03	04	01	02	03	04	01	02	03	04
Ampicillin	0	77	100	100	66	46	67	61	40	0	0	50	7	0	10	6
Co-trimoxazole	0	77	100	100	100	93	78	89	80	66	0	50	100	95	100	100
Tetracycline	0	77	100	100	72	90	92	94	100	33	0	50	100	100	100	100
Nalidixic acid	0	77	100	100	44	46	89	92	60	0	0	0	100	91	100	100
Ciprofloxacin	0	77	100	100	0	3	3·5	41	0	0	0	0	0	0	0	0
Norfloxacin	0	77	100	100	0	3	3·5	41	0	0	0	0	0	0	0	0
Ofloxacin	0	77	100	100	0	3	3·5	41	0	0	0	0	0	0	0	0

Table 2. *Patterns of multidrug resistance in* Shigella *isolates*

Antibiotic resistance profile	S. dysenteriae				S. flexneri				S. boydii				S. sonnei			
	Year				Year				Year				Year			
	01	02	03	04	01	02	03	04	01	02	03	04	01	02	03	04
Am Sxt Na	0	0	0	0	4	5	1	0	0	0	0	0	0	0	0	0
Sxt Te Na	0	0	0	0	2	5	4	7	1	0	0	1	5	20	9	5
Am Sxt Te Na	0	0	0	0	5	4	15	10	2	0	0	0	0	1	1	1
Sxt Te Na Cip Nor Ofx	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0
Am Sxt Te Na Cip Nor Ofx	0	7	6	3	0	1	1	8	0	0	0	0	0	0	0	0

Am, Ampicillin; Sxt, co-trimoxazole; Te, tetracycline; Na, nalidixic acid; Nor, norfloxacin; Cip, ciprofloxacin; Ofx, ofloxacin.

type 1 strains were replaced by various serotypes of *S. flexneri* [22]. Surveillance of the incidence of *Shigella* spp. has clearly shown shifts in species prevalence from time to time in Kolkata which is unlike the situation in Lagos, where *S. flexneri* remains the dominant species [23].

Over the decades, *Shigella* spp. have become resistant to most of the widely used and inexpensive antimicrobials [4, 7]. Almost all the strains in this study were resistant to ampicillin and co-trimoxazole. Before 1990, these agents were the drugs of choice for the treatment of shigellosis [7] and early reports showed that resistance to them was restricted to sporadic and epidemic strains of either *S. dysenteriae* type 1 [24] or *S. sonnei* [25–27]. During 1984, *S. dysenteriae* type 1 strains isolated from a large outbreak in West Bengal exhibited reduced susceptibility to ampicillin and co-trimoxazole. However, these strains were resistant to tetracycline but sensitive to nalidixic acid [28]. Similar resistance profiles were also observed among *S. flexneri* strains isolated in this region in 1987 [29]. In this study, 6–10% of the *S. sonnei* strains

were resistant to ampicillin (Table 1). In Israel, investigations conducted between 1990 and 1995, showed that 75% of *S. sonnei* isolates were resistant to ampicillin and only three strains of *Shigella* spp. were reported as resistant to nalidixic acid [30]. During 1991, ampicillin- and co-trimoxazole-resistant *Shigella* spp. were reported from several parts of the developing world [31–33] whereas in previous years (1985 to 1986), in the United States only 6% of *Shigella* isolates were resistant to both of these drugs [34].

Most of the *Shigella* strains recovered in the present survey were resistant to nalidixic acid. In the early 1990s, nalidixic acid was the drug of choice for treating shigellosis in India [35], Bangladesh [36] and northeastern Brazil [37] and in countries such as Nigeria and Pakistan, nalidixic acid is still used for the treatment of shigellosis [23, 38, 39]. In addition, the re-emergent *S. dysenteriae* strains in Indonesia [18] and most of the *Shigella* strains in Tanzania [40] were reported to be susceptible to nalidixic acid.

A low level of nalidixic acid resistance was first reported in 1988 in a *S. dysenteriae* type 1 outbreak in

the eastern part of India [41]; these strains were also resistant to ampicillin, co-trimoxazole and tetracycline. However, a study conducted in Kolkata showed that *S. dysenteriae*, other than serotype 1, strains were sensitive to nalidixic acid [42] and had resistance patterns similar to those of *Shigella* spp. from Bangladesh and Thailand [31, 33]. During 1995 and 1996, an increase in nalidixic acid-resistant *Shigella* spp. was reported, and with the exception of *S. sonnei* all strains were also resistant to ampicillin [43]. The continued resistance of *S. sonnei* to nalidixic acid reported here is similar to that described recently for Saudi Arabia [44].

After the 1988 *S. dysenteriae* type 1 outbreak, this serotype was not isolated from patients in Kolkata [5] but other *Shigella* spp. acquired resistance to nalidixic acid. Although this antibiotic was recommended in the treatment regime by the WHO [45], the emergence of resistant strains led to use of nalidixic acid being discontinued in Bangladesh and Rwanda [31, 46].

Despite this, fluoroquinolones were extensively used for the treatment of shigellosis, as these drugs provide more effective care [47–49]. During mid-2002, outbreaks of dysentery due to *S. dysenteriae* type 1 were reported [50, 51]. These strains were multidrug resistant, including fluoroquinolones, and clonal in nature [8] and have been reported to spread to Bangladesh and Nepal [52]. In Kolkata, the prevalence of fluoroquinolone resistance is increasing slowly; during 2003, the resistance rate was 15% and by August 2004, it had increased to 25%. This sudden upsurge is due to the emergence of fluoroquinolone resistance amongst predominant endemic isolates of *S. flexneri* particularly serotype 2a, which came to prominence in 2002 (Fig. 2). It is noteworthy that ampicillin-sensitive, fluoroquinolone-resistant strains had also been isolated in Kolkata during August 2004. Recently, it was reported from Bangladesh that *S. flexneri* serotype 2a was predominant but all isolates tested were sensitive to ciprofloxacin [53]. These fluoroquinolone-resistant epidemic strains were not only detected in Kolkata but also reported from Chandigargh in northern India [21].

From this study, it is evident that antimicrobial resistance, particularly to the fluoroquinolones, is increasing year on year among *Shigella* spp. in Kolkata. In a span of 8 years, after fluoroquinolones were introduced for the treatment of shigellosis in this region, resistance has emerged amongst predominant endemic and epidemic strains. This is an ideal example of where unrestricted and indiscriminate use of antimicrobial agents for treating infectious diseases gives rise to the problem of developing drug resistance. If this antimicrobial usage continues, it is predicted that there is a high possibility that *Shigella* spp. might develop resistance against most of the drugs available in this region. In this situation, the treatment options will be severely limited to only a very few antimicrobials such as the macrolide azithromycin or the third-generation cephalosporin, ceftriaxone (S.K.Niyogi, unpublished data). These agents have been reported to be effective against shigellosis in other countries [54–56]. It has also been suggested that the oral antimicrobials azithromycin and pivmecillinam may represent the drugs of choice for the treatment of fluoroquinolone-resistant *S. dysenteriae* type 1 infection [9]. However, the history of *Shigella* spp. suggests that resistance to these agents will emerge and be maintained if they are used extensively. Continued studies should be conducted with special reference to drug resistance to allow timely adaptation of therapeutic recommendations to be made.

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