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Shifting serotypes, plasmid profile analysis and antimicrobial resistance pattern of shigellae strains isolated from Kolkata, India during 1995–2000

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SUMMARY

One hundred and sixty-six shigellae strains, isolated from stool samples of paediatric patients (< 5 years old) at a Childrens' Hospital in Kolkata, India during the period of 1995–2000 were examined for serotyping, drug resistance pattern and plasmid profiles. *Sh. flexneri* (58%) was found to be commonest isolate of total shigellae, followed by *Sh. sonnei* (28%), *Sh. boydii* (9%) and *Sh. dysenteriae* (5%). This profile of species was in sharp contrast to the picture obtained before 1995, when *Sh. dysenteriae* 1 predominated over *Sh. flexneri*. In *Sh. flexneri* strains, *Sh. flexneri* 2a (35%) was the most prevalent serotype, following *Sh. flexneri* 3a (31%), *Sh. flexneri* 6 (14%), *Sh. flexneri* 2b (11%) and *Sh. flexneri* 4 (9%). Resistance patterns of the strains to 12 commonly used antimicrobial agents and minimum inhibitory concentrations (MICs) of the antibiotics were also tested. All strains were found uniformly susceptible to norfloxacin, but more than 90% strains were resistant to tetracycline, co-trimoxazole and 67% strains were resistant to ampicillin. Resistance to amoxicillin, chloramphenicol and nalidixic acid was found in 55% (range 45–74%), 46% (range 40–60%) and 29% (range 15–40%) strains respectively. Overall, shigellae strains showed statistically significant increase in resistance against tetracycline, nalidixic acid and furazolidone ($P < 0{\cdot}05$) over the years of this study. This indicates decreased efficacy of furazolidone, cotrimoxazole and nalidixic acid for the empirical treatment of shigellosis in Kolkata. Although a few strains showed intermediate susceptibility to ciprofloxacin (4%) and cefotaxime (10%) by disk diffusion test, but the MICs of those antibiotics were within the normal limits. Almost 57% of the strains were resistant to four or more drugs with high MICs of the antibiotics. Plasmid profile analysis revealed presence of large plasmid of 220 kb in majority of the strains except in *Sh. sonnei* and a correlation between presence of smaller plasmids and shigellae serotypes. Hence this study reports epidemiological change of *shigellae* species in Kolkata, India with regard to serotypes and antibiotic resistance patterns.

INTRODUCTION

Shigellosis still remains a public health problem in many parts of the world including India not only for morbidity, but also for growth retardation and malabsorption, which are important sequelae to this infection in children [1]. The annual number of shigella episodes throughout the world was estimated to be 164·7 million, of which 163·2 million were in developing countries (including 1·1 million deaths) and 1·5 million in industrialized countries. A total of 69%

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of all episodes and 61% of all deaths attributable to shigellosis involved children below 5 years of age [2].

Changing trend in the epidemiology of shigellosis and antimicrobial resistance pattern of shigellae strains has been noticed throughout the world over last two decades [3–5]. Kolkata, India is not an exception to this. In the year 1984–5, several parts of India including Kolkata experienced a devastating epidemic of shigellosis caused mainly by multiresistant *Sh. dysenteriae* 1 [6, 7]. This *Sh. dysenteriae* 1, the most prevalent serotype of 1990s, was totally replaced with *Sh. flexneri* during 1995–6 and high-level antibiotic resistance was also developed in isolated strains [8]. In contrast, in Andaman, a tropical island in the Bay of Bengal, simultaneous isolation of multidrug resistant *Sh. dysenteriae* and *Sh. flexneri* was observed during 1994–6 [3]. Predominance of *Sh. flexneri* still continues in several parts of developing world, but in industrialized countries *Sh. sonnei* remained the most commonly isolated strain [2, 4].

Shigella is known to harbour a number of plasmids, of which the heavier plasmids of 160–220 kb play an important role in tissue invasion [9]. Only a few studies documented usefulness of plasmid profiles for investigation of an outbreak caused by this organism [10]. Antimicrobial resistance pattern, plasmid profile and serotype correlation of shigellae strains was reported from several countries [11–13]. But no such study has been reported from India.

Although WHO recommends early antibiotic therapy for treatment of any shigellosis cases [14], it cannot be controlled adequately with existing treatment and control measures. Emergence of multidrug resistant strains further complicated the situation [5, 8, 15]. Hence, developing a vaccine against the most prevalent strain may be a suitable alternative for prevention of this disease. Therefore, any shift in the trends of seroprevalence and antimicrobial resistance patterns of shigellae strains needs to be monitored very carefully in any geographical region.

Considering the background information, in the present study, we intend to determine the serotype prevalence and to monitor the antibiotic resistance pattern of shigellae strains isolated from children hospitalized due to acute diarrhoea in Kolkata, India during May 1995 to November 2000. Attempts have also been made to determine any correlation between plasmid profiles and serotype patterns of isolated shigellae strains.

MATERIALS AND METHODS

Study population

A total of 2855 children under 5 years old suffering from acute diarrhoea irrespective of type, duration and severity of the disease, without having any history of drug therapy, who were admitted to Dr B. C. Roy Memorial Children Hospital, Kolkata between May 1995 to November 2000 were included in this study (Table 1). Dr B. C. Roy Memorial Hospital for children, Kolkata is the biggest paediatric hospital in Kolkata, providing free treatment to inpatients and outpatients. Catchment area of this hospital includes Kolkata metropolis and suberbs. Patients come from all socioeconomic groups either directly or after referral from other hospitals. Approximately 8000–10000 diarrhoea patients are admitted to the hospital annually. Another 15000–18000 diarrhoea patients receive treatment from outpatients departments. A surveillance system was set up by National Institute of Cholera and Enteric Diseases, Kolkata to study a representative sample of diarrhoea patients at this hospital.

Sample collection

Fresh stool samples were collected from the patients after admission and transported to the microbiology department of the Institute for further testing. Stool samples were processed within 2 h of submission by both conventional microbiological and molecular biological methods for identification of established enteric bacterial, viral and parasitic pathogens. The samples, which yielded shigellae strains of known serotypes as a sole pathogen, were the subjects of this communication.

Microbiological methods

Stool specimens were examined microscopically for presence of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* species. Presence of rotavirus antigen was detected by running PAGE of the samples [16]. Identification of bacterial pathogens, e.g. *V. cholerae*, *V. parahaemolyticus*, *Campylobacter* spp., *Aeromonas* spp., *Salmonella* spp. was performed employing standard conventional methods [17] and *diarrhoeagenic Escherichia coli* was differentiated by molecular biological techniques [18].

Table 1. *Isolation of shigellae from stool samples of hospitalized children with acute diarrhoea in Kolkata from 1995–2000*

Year	No. of samples tested	No. of samples positive for shigella (%)	Serogroup distribution in shigella positive samples (%)			
			SD	SF	SB	SS
1995	333	13 (3·9)	2 (0·6)	6 (1·8)	3 (0·9)	2 (0·6)
1996	585	34 (5·8)	3 (0·5)	18 (3·1)	7 (1·2)	6 (1·0)
1997	316	18 (5·7)	0	9 (2·8)	0	9 (2·8)
1998	342	20 (5·8)	0	14 (4·1)	2 (0·5)	4 (1·1)
1999	678	46 (6·8)	1 (0·1)	28 (4·1)	0	17 (2·5)
2000	600	35 (5·8)	3 (0·5)	21 (0·3)	2 (0·3)	9 (1·5)
Total	2855	166 (5·8)	9 (0·3)	96 (3·4)	14 (0·5)	47 (1·6)

* SD, *Sh. dysenteriae*; SF, *Sh. flexneri*; SB, *Sh. boydii*; SS, *Sh. sonnei.*

Table 2. *Antimicrobial resistance pattern of isolated shigella strains from Kolkata during 1995–2000 (percentage)*

Antibiotic disk (symbol)	Disk content (μg/disk)	1995 ($n = 13$)	1996 ($n = 34$)	1997 ($n = 18$)	1998 ($n = 20$)	1999 ($n = 46$)	2000 ($n = 35$)
Ampicillin (I)	10	69	74	100	100	42	57
Tetracycline (T)	30	100	100	89	100	98	80
Chloramphenicol (C)	30	46	50	45	60	44	34
Co-trimoxazole (Q)	25	100	100	100	100	94	97
Furazolidone (Fz)	100	16	9	0	0	20	71
Nalidixic acid (NA)	30	15	38	0	15	37	40
Ciprofloxacin (CI)	5	0	0	0	0	2	17
Norfloxacin (NF)	10	0	0	0	0	0	0
Gentamicin (G)	10	0	0	5	5	0	3
Amikacin (AK)	30	0	0	0	10	6	57
Cefotaxime (CX)	30	0	0	22	10	11	14
Amoxycillin (Amx)	10	62	74	56	60	48	46

Identification of *Shigellae* species

Selective enrichment method was used for isolation of shigellae strains from stool samples. Each sample was inoculated onto MacConkey agar (MAC), Xylose lysine Desoxycholate agar (XLD), and Hectoen Enteric agar (HEA) after enriching the sample in shigella broth for 4–6 h. After overnight incubation at 37 °C specific colonies were identified as *Shigellae* species by biochemical tests and using API 20E strips (Biomauriex Foundation). The identification was confirmed serologically by slide agglutination with commercially available group and type specific antisera (Denka Seiken Co., Japan). All isolated shigellae strains were examined for antimicrobial susceptibility to a panel of 12 antibiotics (Table 2) using commercially available antibiotic disks (Becton Dickinson, USA). The analyses were done by disk diffusion and *Escherichia coli* ATCC 25922 was used as control strain for quality control assessment of antibiotic disks. The strains were designated as resistant (this also included intermediate sensitive strains) and sensitive based on the size of zone of inhibition given with manufacturer's instructions. Minimum Inhibitory Concentrations (MICs) of the antibiotics, to which the strains were resistant or intermediate sensitive by disk diffusion method, were determined by agar dilution technique [19].

Plasmid profiles of isolated shigella strains

Luria Broth (Difco, USA) was used to grow bacteria for plasmid DNA extraction. Representative strains of each shigellae serogroup were selected from isolated shigellae strain lots and plasmids were extracted by the standard method [20]. The plasmids were analysed by 0·8% agarose gel electrophoresis, strained with Etbr solution and compared. A suitable DNA size marker (*E. coli* V517) was run in each gel to obtain the

size of each of the plasmids of test shigellae strains [21].

Statistical analysis

The proportion of isolates of each shigellae serotypes and their antimicrobial susceptibility results were compared by the χ^2 and Fisher's exact tests. The data were entered in a personal computer, checked randomly for validation and then compiled for analysis in SPSS 4.0 version software package. A probability value $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

Stool samples from 2855 patients were processed during the entire study period, of which 1998 (70%) had acute watery diarrhoea and 857 (30%) patients had mucoid diarrhoea with or without blood. Standard culture techniques detected bacterial, viral and parasitic enteropathogens in 1714 (60%) diarrhoeal children, which included mixed pathogens in 257 (9%) patients. The sole enteropathogens detected were rotavirus (7%), *Salmonellae* spp. (3%), *V. cholerae* (2·5%), *Campylobacter* spp. (2%), *Aeromonas* spp. (3%), *Cryptosporidium* (2%), *Giardia lamblia* (0·7%), *Ent. histolytica* (0·8%) and enterovirulent *Escherichia coli* (24%).

Shigellae species were isolated as sole pathogen from 166 (6%) children by the conventional method. Out of 166 shigellae positive patients, 33 (20%) had watery stool, 83 (50%) complained of blood mucoid diarrhoea and 50 (30%) children had only mucoid diarrhoea without blood. Isolation rate of shigellae was significantly higher (10%) among children of 13–60 months as compared to only 2% in children aged less than 6 months ($P < 0{\cdot}05$). Male children were more affected than females, male : female ratio being 1·4 : 1. The majority (80%) of the children belonged to the low socio-economic class with poor personal hygiene.

Table 1 shows the number of shigellae in each serogroup identified during each year in 1995–2000. *Sh. flexneri* (SF) was the most predominant type isolated from 58% (96/166) of all shigellosis cases followed by *Sh. sonnei* (SS) from 28% (47/166) cases, *Sh. boydii* (SB) from 9% (14/166) and *Sh. dysenteriae* (SD) from 5% (9/166) cases respectively.

Figure 1 is a 100%-stacked column bar chart showing relative percentage distribution of different shigellae serogroups over the last eight years (1993–2000). During 1993–4, the median percentages of

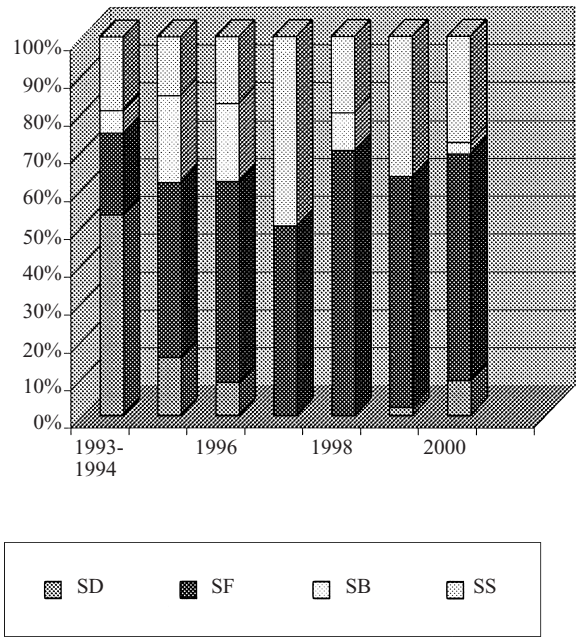

Fig. 1. 100%-stack column bar chart showing isolation frequency of different shigella serotypes over the years.

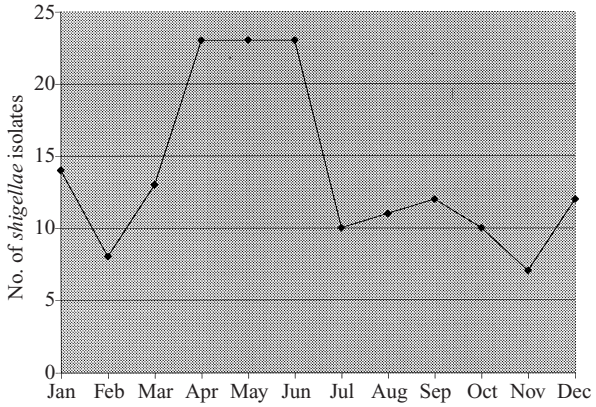

Fig. 2. Monthly isolation of all shigella serotypes from diarrhoeal children over 6 years.

isolates of *Sh. dysenteriae*, *Sh. flexneri*, *Sh. sonnei* and *Sh. boydii* were, 53%, 22%, 20% and 5% respectively (unpublished data). In sharp contrast, in 1995 *Sh. flexneri* (46%) over took *Sh. dysenteriae* (15%), and this trend continued thereafter until recently.

Among *Sh. flexneri* strains, *Sh. flexneri* type 2a (SF2a) was the commonest subtype (35%). Other common serotypes included *Sh. flexneri* type 3a (31%), *Sh. flexneri* 6 (14%), *Sh. flexneri* 2b (11%) and *Sh. flexneri* 4 (9%).

Figure 2 illustrates the overall monthly isolation of all *Shigellae* species during 6 calendar years. Although no predilection for specific months of the year was noted with shigellae isolation, in the pre-monsoon months of April–June an increased number of

Table 3. *MICs of various antimicrobials for shigella strains from Kolkata*

Antimicrobials	Mean (range) in μg/ml MIC_{90}
Ampicillin	128 (128–512)
Tetracycline	128 (64–256)
Chloramphenicol	64 (64–256)
Co-trimoxazole	64 (32–64)
Furazolidone	64 (32–128)
Nalidixic acid	64 (32–128)
Amikacin	64 (64–128)
Gentamicin	64 (64–128)
Ciprofloxacin	0·125 (0·0625–0·25)
Cefotaxime	< 0·0625

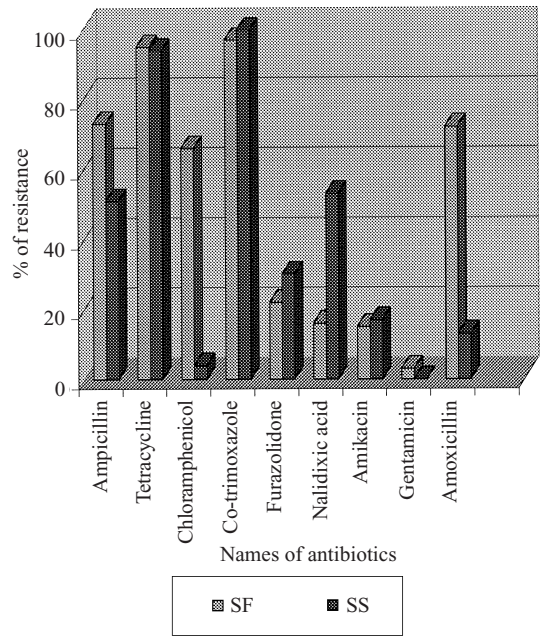

Fig. 3. Comparison of antimicrobial resistance of *Sh. flexneri* and *Sh. sonnei* strains from Kolkata.

microbiologically confirmed shigellosis cases was admitted to the hospital.

Table 2 shows antimicrobial resistance patterns of all shigellae strains isolated during the period of study. There was no evidence of resistance to norfloxacin, a fluoroquinolone derivative. But uniform resistance to amoxicillin, chloramphenicol, co-trimoxazole and gradual increase in resistance to tetracycline, furazolidone and nalidixic acid was noted over the years. In 1998–2000 shigella strains also developed resistance to amikacin, to which they were susceptible earlier.

Table 3 documents the minimum inhibitory concentrations (MICs) of the antibiotics, to which the organism was found to be resistant. The MIC values revealed that more than 90% shigellae strains had high-level resistance to ampicillin, tetracycline and cotrimoxazole, and were moderately resistant to chloramphenicol, nalidixic acid and furazolidone. During recent years, a few strains showed intermediate sensitivity to ciprofloxacin (7 strains) and cefotaxime (16 strains) by the disk diffusion method, but MICs of those antibiotics were found to be 0·125 μg/ml and < 0·0625 μg/ml respectively, which was within normal limits.

Table 4. *Drug resistance R profiles of shigella strains during 6 years*

R profiles*	No (%) of strains (n = 166)
ICTQ	65 (39)
ITQ	40 (24)
ITQNA	30 (18)
ICTQNA	18 (11)
ICTQAK	7 (4·2)
ICTQNAG	4 (2·4)
IQ	2 (1·2)

* I, ampicillin; T, tetracycline; C, chloramphenicol; Q, co-trimoxazole; NA, nalidixic acid; AK, amikacin; G, gentamicin.

Figure 3 compares the drug resistance patterns of *Sh. flexneri* and *Sh. sonnei*, the two most prevalent strains in Kolkata during the period of study. Since isolation of *Sh. dysenteriae* (0·3%) and *Sh. boydii* (0·05%) strains was negligible as compared to total diarrhoea cases (Table 1), they were not included in the analysis.

Table 4 indicates distribution of common R patterns of all shigellae strains during the 6 year period. It was observed that almost 57% strains were resistant to four drugs. Common drug profiles associated with shigellae strains were ICTQ (39%), ITQ (24%) and ITQNA (18%). But the resistance profile was not constant for any specific serotype and similar profile was observed in different serotypes.

Figures 4 and 5 depict plasmid profiles of various shigellae strains. It was observed that large plasmids (approximately 220 kb) were present in almost all *Sh. flexneri*, *Sh. dysenteriae* and *Sh. boydii* strains, whereas they were absent in *Sh. sonnei*. Almost all shigellae strains possessed multiple copies of smaller plasmids. All *Sh. flexneri* strains had plasmids of 3·9 kb and 5·7 kb size, whereas all *Sh. sonnei* strains showed the presence of 2·7 kb and 6·5 kb plasmids and the absence of 3·9 kb and 5·7 kb plasmids.

For comparing plasmid profiles of each serogroup, the large plasmids have been ignored. The smaller

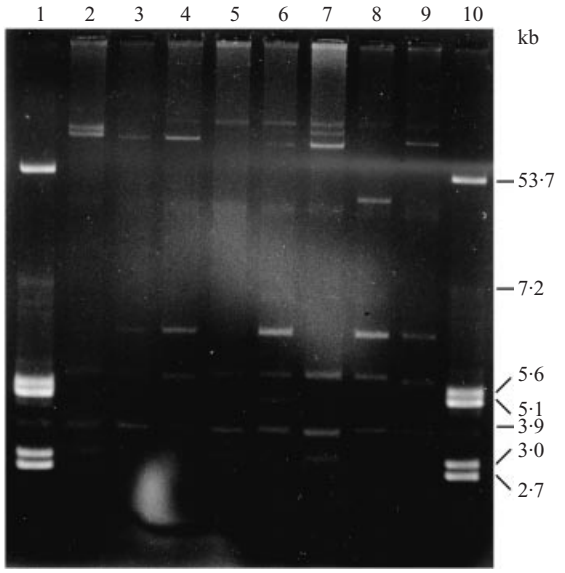

Fig. 4. Plasmid profiles of *Sh. flexneri* and *Sh. dysenteriae* strains isolated solely from children with acute diarrhoea after agarose gel (1%) electrophoresis of plasmid DNA of the strains. Lanes 1 and 10, *E. coli* V517 (DNA size marker) [21]; Lane 2, *Sh. flexneri* 2a (isolated in 1997); Lane 3, *Sh. flexneri* 4 (isolated in 1997); Lane 4, *Sh. flexneri* 4 (isolated in 2000); Lane 5, *Sh. flexneri* 2a (isolated in 2000); Lane 6, *Sh. flexneri* 4 (isolated in 2000); Lane 7, *Sh. flexneri* 2a (isolated in 2000); Lane 8, *Sh. dysenteriae* 2 (isolated in 2000); Lane 9, *Sh. dysenteriae* 2 (isolated in 2000).

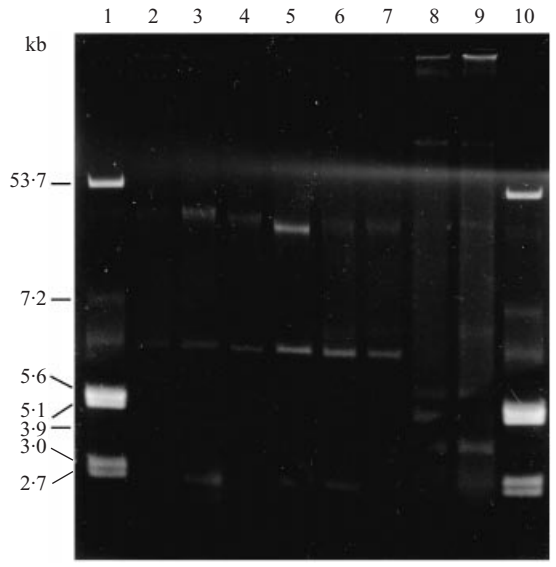

Fig. 5. Plasmid profiles of *Sh. sonnei* and *Sh. boydii* strains isolated solely from children with acute diarrhoea after agarose gel (1%) electrophoresis of plasmid DNA of the strains. Lanes 1 and 10, *E. coli* V517 (DNA size marker) [21]; Lane 2, *Sh. sonnei* (isolated in 1997); Lane 3, *Sh. sonnei* (isolated in 1999); Lanes 4–7, *Sh. sonnei* (isolated in 2000); Lane 8, *Sh. boydii* strain (isolated in 2000), Lane 9, *Sh. boydii* (isolated in 2000).

plasmids were found to be group specific and could be used as marker plasmids.

DISCUSSION

Shigellosis is the major cause of dysentery in children and *Sh. flexneri* is the commonest strain responsible for the disease in most developing countries including India. Studies from Kolkata documented a preponderance of *Sh. flexneri* strains during 1984–7 [22], but later *Sh. dysenteriae* 1 became the most predominant strain (56%) during 1990–2 [23] and the predominance continued until the present study period. This indicates the dynamic nature of this organism in this part of India. After 1995, again an increasing prevalence of *Sh. flexneri* was noted throughout the study period, and this varied from 46 to 70% (mean $16 \pm$ S.D. 8·1). Concomitantly, the prevalence of *Sh. dysenteriae* came down from 16% in 1995 to below 10% in 2000 (mean $1{\cdot}5 \pm$ S.D. 1·4). This difference in isolation rate was statistically significant ($P < 0{\cdot}05$). *Sh. sonnei* remained the second commonest serotype with isolation ranging from 15% to as high as 50% (mean $7{\cdot}8 \pm$ S.D. 5·3) (Table 1).

It appears that Calcutta and its suburbs became the endemic zone for *Sh. flexneri* after the epidemic of *Sh. dysenteriae* type 1 in 1984 excluding a short period of SD1 predominance during 1990–4. The reason for replacement of *Sh. dysenteriae* by *Sh. flexneri* is unknown, though the living and environmental conditions of the people of this region remained same for many years. The relative survival advantage of *Sh. flexneri* over *Sh. dysenteriae* in the environment may be a plausible explanation for endemicity of *Sh. flexneri* strains and disappearance of *Sh. dysenteriae* strains. But this hypothesis is yet to be proved.

The predominant serotypes identified were *Sh. flexneri* type 2a (35%) and *Sh. flexneri* type 3a (31%). A similar observation was also reported in previous studies from Kolkata [8]. There are reports documenting *Sh. flexneri* 2a as the predominant serotype in developing countries followed by 1b, 3a, 4a and 6 [2, 3]. Knowledge of the predominant shigellae serotypes of any region has immense importance because it helps in selecting the type-specific antigen for development of a candidate vaccine provisionally used for that region.

Antibiotics are always recommended for the treatment of childhood shigellosis [14]. Hence, monitoring the antibiotic resistance profile of circulating shigellae strains is very important in selecting the drug of choice

for shigellosis. Nalidixic acid was the drug of choice for the treatment of shigellosis, but due to emergence of resistant strains from various part of the country, recently norfloxacin or ciprofloxacin (fluroquinolones) have been used in day-to-day practice, although with caution in children [24].

Uniform sensitivity (100%) of all shigellae isolates to norfloxacin was observed throughout the study period. But a gradually increase in multidrug resistance was noted against commonly used antibiotics such as ampicillin (67%), tetracycline (93%), chloramphenicol (46%), co-trimoxazole (98%), furazolidone (25%), nalidixic acid (29%) and amoxicillin (55%) and in most of the cases the increase was statistically significant ($P < 0{\cdot}05$). More than 57% of the shigellae strains were resistant to four or more antibiotics. This finding corroborates the report of earlier studies from Kolkata [8, 23] and also studies from other geographical regions [3, 15].

Serotype specific resistance pattern reveals that in recent years although *Sh. sonnei* developed more resistance to nalidixic acid and furazolidone, an overall increased resistance to ampicillin, amoxicillin and chloramphenicol was observed in *Sh. flexneri* strains. Resistance to nalidixic acid increased from 15% to 40% ($P < 0{\cdot}05$) for shigellae strains over the years, which was due to acquisition of resistance mainly by *Sh. sonnei* from 33% in 1996 to 100% in 2000 (av. 53%), whereas it remained more or less the same for *Sh. flexneri* at 16% in 1995 and 19% in 2000 (av. 16%). This difference in resistance in SF and SS strains was statistically significant. Moreover, *Sh. sonnei* were 100% susceptible to amplicillin during 1995–6, but 100% were resistant in 1997–8 and 55% were resistant in 2000. An increase in resistance to furazolidone and amikacin was also observed for *Sh. flexneri* since 1998 and for *Sh. sonnei* since 1999 onwards and in both cases the increase was statistically significant ($P < 0{\cdot}05$).

All shigellae strains were susceptible to ciprofloxacin, gentamicin, amikacin and cefotaxime in 1995–6, but since 1997 onwards a few shigellae strains have been found intermediate, susceptible to ciprofloxacin and cefotaxime by the disk diffusion method. MICs determination of ciprofloxacin (MIC_{90} 0·125) and cefotaxime ($MIC_{90} < 0{\cdot}0625$) indicated that their values were within normal limits. This result raised doubt about usefulness and validity of the disk diffusion test for determining susceptibility to ciprofloxacin and cefotaxime by using commercially available disks.

Plasmid profiles of microorganisms have long been used as reliable and useful tool for strain discrimination in various epidemiological studies [12, 13], for which plasmid bands of less than 20 kb were usually compared.

In this study, all isolated *Sh. flexneri* strains showed presence of plasmid bands of 3·9 and 5·7 kb size. Apart from those, a smaller band 3·1 kb size was also found in almost all *Sh. flexneri* 2a strains. This 3·1 kb plasmid was absent in *Sh. flexneri* 4 strains, instead, they harboured a plasmid of 6·3 kb. Two *Sh. dysenteriae* 2 strains, isolated during this period, were almost identical in their plasmid pattern (Fig. 4). Two *Sh. boydii* strains also showed similar plasmid profiles (Fig. 5). This indicated that each shigellae serogroups of Kolkata was associated with a distinct plasmid profiles and this association has remained uniform for the last 6 years. To our knowledge, this is the first report from this region indicating an association of distinct plasmid profiles with shigellae serogroups. A similar observation was also reported from other geographical regions [10–13].

Although plasmid bands with an apparently identical size were present in more than one serogroup, the characteristic plasmid profile of each serogroup with minor variation may be helpful in detecting and tracing serologically untypable strains (Sh OUT, not agglutinated by commercially available antisera). A number of such strains have already been reported from Kolkata [25], which may acquire new epidemiological importance in future. There are reports, which documented an association of untypable *Sh. flexneri* strain with an outbreak in California [26] and the isolation of untypable *Sh. dysenteriae* strains from Dutch travellers who visited India [27]. One more study from Bangladesh also reported emergence of serologically atypical *Sh. flexneri* 1c and 4x strains in Dhaka [28].

From this observation it may be concluded that long-term surveillance programme is essential, where shigellosis is endemic, to determine any change in serotype prevalence, emergence of new serotypes and to monitor the antibiotic resistance profiles of shigellae strains. The profiles of small plasmids of local strains can be used as a marker to identify known serotypes or to determine the emergence of any new and unknown serotype in future. In view of the emergence of the multidrug resistant strains, suitable alternative drugs should be sought for the treatment of childhood shigellosis, as the use of fluroquinolones in all paediatric age groups is still controversial. The

spread of shigellosis in the community may be controlled with improved personal hygiene, but it is very difficult to achieve this in developing countries. Hence, development of vaccines against the most common serotypes could provide a suitable alternative for containment of the disease.

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