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SHORT REPORT
Shigella serotypes among hospitalized patients in urban Bangladesh and their antimicrobial resistance

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SUMMARY

We studied the isolation of *Shigella* spp., and their antimicrobial resistance. *S. flexneri* (54%) was most frequently isolated, followed by *S. dysenteriae* (20%), *S. boydii* (16%) and *S. sonnei* (10%). Among *S. flexneri* ($n=122$), 29 (24%) were 2a, and 23 (19%) were 2b. None of the *Shigella* strains were resistant to mecillinam or ciprofloxacin. Resistance to nalidixic acid was most frequent among *S. dysenteriae* type 1 (100%) followed by *S. flexneri* 2a (69%), and *S. flexneri* 2b (52%). Systematic monitoring is needed to identify most prevalent serotypes, and to detect changes in the prevalence and antimicrobial resistance pattern.

Despite gradual improvements in water supply and sanitation, shigellosis continues to be endemic among the disadvantaged populations living in the tropics. Additionally, *Shigella* epidemics often occur among displaced populations following natural disasters and political crisis [1–5]. Emergence of multiply-resistant strains of *Shigella* is a great public-health problem since antimicrobial therapy, effective in reducing morbidity and deaths, is routinely indicated in the management of shigellosis [6]. In endemic countries, shigellosis is primarily a disease of children, and may be caused by any of the four species of *Shigella*, namely, *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* [4]. Each of these species, with the exception of *S. sonnei* that has only one serotype, is further subdivided into serotypes and subserotypes. Based on biochemical reactions or lipopolysaccharide characteristics of the strains at least 47 serotypes are currently recognized [7, 8]. In endemic countries, the majority of infections are caused by *S. flexneri*, and large epidemics and pandemics are often caused by multiply-resistant strains of *S. dysenteriae* type 1, which is also endemic in some developing countries including Bangladesh [9]. We identified patients admitted to the Dhaka Hospital of ICDDR,B with *Shigella* infections between January 2000 and September 2001, and reviewed the distribution of *Shigella* and their antimicrobial resistance by serogroups as well as serotypes and subserotypes. We believe that the results of our study will help clinicians in the selection of appropriate antimicrobial agents in the management of shigellosis, and will also serve as reference for comparison in future studies in Bangladesh and elsewhere.

All consecutive patients (or parents/guardians in the event the patient was a child), regardless of their age and sex, who attended the hospital between 06:00 and 19:30 hours each day of the week for treatment of their diarrhoeal illness of <96 h duration and who had a history of presence of visible blood and/or mucus in stool were interviewed for collection of relevant information. Those clinically suspected as possible cases of shigellosis were identified, and their stool specimen was collected for microscopic examination and culture. Patients, who had received antimicrobial therapy for their illness, before attending the hospital, were excluded from the study. Informed

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consent was obtained from the patients or their legal guardians as appropriate.

Patients attending the hospital with history of presence of blood and/or mucus in at least one loose stool in the previous 24 h, and >10 faecal leucocytes with any number of erythrocytes per high power field in the microscopic examination of their freshly collected stool specimen were considered as presumptive cases of shigellosis. A culture-confirmed case was defined as a patient who had *Shigella* isolated from a stool/rectal swab culture either as a single or mixed pathogen.

Trained health staff collected data from a reliable family member using pre-tested forms and a physician obtained the medical history, performed a physical examination, and recorded the findings in prescribed forms.

Freshly collected stool specimens from the patients were inoculated onto MacConkey and Salmonella–Shigella agar media plates. *Shigellae* were isolated and identified using standard laboratory methods [10].

Identification and serotyping of *Shigella* were performed using commercial antisera kits (Denka Seiken, Tokyo, Japan) specific for polyvalent and monovalent antigens for all serovars of *Shigella*. For serotyping of *S. flexneri*, a panel of monoclonal antibodies specific for all *S. flexneri* type- and group-factor antigens (Reagensia AB, Stockholm, Sweden), was used. Strains were subcultured on MacConkey agar (Difco, Becton Dickinson & Company, Sparks, MD, USA) plates, incubated for approximately 18 h, and serotyping was performed by slide agglutination test as described earlier [11].

Antimicrobial susceptibility of *Shigella* strains was determined by the disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1990) using commercial antimicrobial discs (Oxoid, Basingstoke, UK). The antibiotic discs used in this study included ampicillin (10 μg), tetracycline (30 μg), mecillinam (25 μg), nalidixic acid (30 μg), trimethoprim–sulphamethoxazole (T-S) (25 μg), and ciprofloxacin (5 μg). *E. coli* ATCC 25922 was used as control strain for susceptibility studies [12].

Data were entered onto a computer and analyses were performed using SPSS for Windows (version 10.2; SPSS Inc., Chicago, IL, USA).

As shown in Table 1, *S. flexneri* was the most frequently isolated species ($n=122$; 54%) followed by *S. dysenteriae* (20%), *S. boydii* (16%) and *S. sonnei* (10%). Among *S. flexneri* ($n=122$), 29 (24%) were 2a, 23 (19%) were 2b, 21 (17%) were type 1 (1a, 1b and 1c), 16 (13%) were 3a, 13 (11%) were 4X (new subserotype), [7, 8, 11] and 9 (7%) were type 6. Among *S. dysenteriae* serogroup ($n=45$), *S. dysenteriae* type 2–7 were more frequently isolated ($n=27$; 60%) than *S. dysenteriae* type 1 ($n=8$; 18%) serotype.

Table 1. *Distribution of* Shigella *spp. and their serotypes*

Organism	*n* (%)
Shigella dysenteriae	45 (20)
S. dysenteriae (1)	8 (3)
S. dysenteriae (2–7)	27 (12)
S. dysenteriae (8–12)	10 (4)
Shigella flexneri	122 (54)
S. flexneri type 1	21 (9)
S. flexneri 2a	29 (13)
S. flexneri 2b	23 (10)
S. flexneri 3a	16 (7)
S. flexneri 4X	13 (6)
S. flexneri type 6	9 (4)
S. flexneri X	1 (0·4)
S. flexneri rough	2 (1)
S. flexneri serotype not done	8 (4)
Shigella boydii	38 (16)
S. boydii (1–7)	9 (4)
S. boydii (8–11)	15 (7)
S. boydii (12–15)	12 (5)
S. boydii (16–18)	2 (1)
Shigella sonnei	22 (10)
Total	227

Antimicrobial susceptibility of all strains of *Shigella* ($n=227$) were determined against five commonly used agents: ampicillin, T-S, nalidixic acid, mecillinam and ciprofloxacin (Table 2). Resistance to nalidixic acid was the highest among *S. dysenteriae* type 1 (100%), followed by *S. flexneri* 2a (69%), and *S. flexneri* 2b (52%). Among all strains of *S. flexneri*, 74% were resistant to T-S, 66% to ampicillin, and 34% to nalidixic acid. All strains (100%) of *S. dysenteriae* type 1, 55% of the *S. sonnei* strains and only 8% of the *S. dysenteriae* type 2–12 strains were resistant to nalidixic acid. Among *S. flexneri*, the type 2a serotype strain was more frequently resistant to nalidixic acid (the current first-line drug at the study site), than the *S. flexneri* other subserotypes (69 *vs.* 9%; $P<0{\cdot}001$), and 52% of the *S. flexneri* 2b isolates were resistant to this drug (Table 2). Shigellosis remains an important cause of childhood illnesses and deaths, and is also a leading cause of malnutrition in Bangladesh [13–16].

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Table 2. *Antimicrobial resistance pattern of* Shigella *isolates*

Species and serotype	No. (%) tested	% Resistant				
		Amp	T-S	Nal	Mec	Cip
S. flexneri	122 (54)	66	74	34	0	0
S. flexneri type 1	21	86	62	5	0	0
S. flexneri 2a	29	86	90	69	0	0
S. flexneri 2b	23	96	87	52	0	0
S. flexneri 3a	16	0	69	31	0	0
S. flexneri other subserotype	33	49	73	9	0	0
S. dysenteriae	45 (20)	42	73	24	0	0
S. dysenteriae (1)	8	100	100	100	0	0
S. dysenteriae (2–12)	37	30	68	8	0	0
S. boydii	38 (16)	34	58	11	0	0
S. sonnei	22 (10)	9	91	55	0	0
All *Shigella*	227	51	74	30	0	0

Amp, Ampicillin; T-S, co-trimoxazole; Nal, nalidixic acid; Mec, mecillinam; Cip, ciprofloxacin.

It emerged as a major public-health problem in Bangladesh following the 1971 war of liberation [2]. The situation worsened in 1972 and 1973 when *Shigella* accounted for 15–30% of hospitalized cases (during the non-cholera season) in urban Bangladesh [17]. Subsequently, *Shigella* appeared as one of the four (rotavirus, ETEC, *V. cholerae* and *Shigella*) common diarrhoeal pathogens among rural children [18]. A number of studies have demonstrated that similar to other developing countries all four serogroups of *Shigella* spp. co-existed in various proportions in Bangladesh [19, 20]. In our study, *S. flexneri* accounted for 54% of all *Shigella* followed by *S. dysenteriae* (20%), *S. boydii* (16%) and *S. sonnei* (10%) signifying that *S. flexneri* is the leading species in Bangladesh, which is similar to other developing countries. Kotloff et al. [21] reported that *S. flexneri* 2a was the predominant serotype in developing countries followed by *S. flexneri* 1b, 3a, 4X and 6. In developed countries, most *S. flexneri* strains are *S. flexneri* 2a or other unspecified type 2 strains. In Bangladesh, Talukder et al. [11] noted a prevalence of *S. flexneri* 2b of 23%, followed by 2a (16%), 3a (16%), 1b (15%), and 1c (8%).

In 1973, *S. flexneri* isolates were universally susceptible to ampicillin; however, by 1979 susceptibility decreased to 79% in urban Bangladesh. Similarly, the susceptibility of *S. flexneri* to tetracycline dropped from 79% in 1973 to 15% in 1979 [20]. A striking increase in the prevalence of multiply-resistant *Shigella* strains has been reported from Bangladesh [9, 22–25]. In 1983, 13% of *Shigella* isolates were resistant to ampicillin and 24% to T-S when ampicillin was the drug of choice for empiric treatment of shigellosis [9]. Because of the increasing resistance to ampicillin and T-S, nalidixic acid was introduced as the drug of choice in 1986 [24–26]. By 1988, 58% of *Shigella* isolates became resistant to ampicillin, 44% to T-S, and 37% to both ampicillin and T-S [9]. The susceptibility of all *Shigella* to nalidixic acid decreased from 100% in 1986 to 80% in 1990, and the resistance pattern differed by serogroup – the highest resistance was observed among *S. dysenteriae* type 1 strains than any other serotypes of *Shigella* [9, 27–29]. In 1990, 69% of the *S. dysenteriae* type 1 isolates were resistant to T-S, 72% to ampicillin, and 68% to both ampicillin and T-S. Among *S. dysenteriae* type 1 strains, resistance to nalidixic acid increased from 2% in 1986 to 58% in 1990 [9]. *S. flexneri* isolates were more commonly resistant to ampicillin, T-S, and to both agents compared to *S. boydii*, *S. sonnei* and *S. dysenteriae* 2–10 strains. In our study, at least 25% of *S. flexneri* were resistant to three commonly used antibiotics such as, ampicillin, T-S and nalidixic acid, which is much higher than has been observed in the studies performed in Bangladesh in the early 1980s, but comparable with recent reports from Bangladesh. The findings thus clearly demonstrate that *Shigella* spp. are becoming increasingly resistant to the commonly used antimicrobials [30, 31].

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In Bangladesh, the resistance of *Shigella* is most common to the combinations of ampicillin, T-S and nalidixic acid. Multiply-resistant *Shigella* spp. have been observed for over two decades [29, 31]. In developing countries, the most common pattern of resistance is to four or more antibiotics such as tetracycline, chloramphenicol, ampicillin, sulphonamides and streptomycin – the drugs that are often used in the treatment of *Shigella* infections. This is believed to be related to the inappropriate use of antimicrobial agents that is facilitated by over-the-counter sales of drugs – a persistent problem in Bangladesh [32, 33].

There are a number of limitations of our study. First, our study was restricted to patients presenting to a hospital with bloody and/or mucoid stool, and they are likely to represent cases with more severe infections and thus may not be representative of the general population. It is possible that this selection bias resulted in a higher proportion of multiply-resistant strains than in the community. Secondly, this study was hospital based, so all subjects who might have developed the disease due to differences in environmental exposures might not have been properly included into the study. Our exclusion of culture-negative invasive diarrhoea episodes might have excluded some actual cases of *Shigella* infections since culture methods are known to have some limitations.

We described the recent pattern of distribution of various species and serotypes of *Shigella* spp. in Bangladesh and their antimicrobial susceptibility patterns. The information may be useful in the clinical management of patients with shigellosis in Bangladesh and other developing countries. The results of our study and earlier studies clearly demonstrate the need for systematic monitoring of the changes in the distribution of various species and serotypes of *Shigella*, and their antimicrobial susceptibility patterns. The findings also signify the need for in-depth epidemiological studies to understand the factors that determine the changes in the pattern of distribution of *Shigella* spp. and serotypes and antimicrobial resistance, and the implications of the distribution among different sex and various age groups. Finally, the results of previous studies and our study, particularly the increasing antimicrobial resistance, strongly suggests the need to identify effective and sustainable strategies to prevent *Shigella* infections including the development of effective and low-cost vaccines that would be affordable to use in developing countries.

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