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Bacterial aetiology of diarrhoeal diseases and antimicrobial resistance in Dhaka, Bangladesh, 2005–2008

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SUMMARY

Infectious diarrhoea caused by bacterial pathogens contributes to the high level of mortality in developing countries like Bangladesh. Following standard bacteriological procedures, a total of 14 428 bacterial pathogens were isolated from 56 132 stool samples and rectal swabs collected from diarrhoeal patients between 2005 and 2008. The rate of isolation and antimicrobial susceptibility data were retrospectively analysed for these isolates and among them *Vibrio* spp. (42·9%) were the most predominant, followed by *Shigella* spp. (20·3%), *Aeromonas* spp. (12·8%) and *Salmonella* spp. (6·4%). A decreasing trend in isolation of *Vibrio* spp. ($P<0·001$) and *Salmonella* spp. ($P<0·001$) was observed. While *Vibrio cholerae* isolates remained susceptible to ciprofloxacin, an increase in resistance was observed in *Campylobacter* spp. and *Shigella flexneri*. Variations in susceptibility to other tested antibiotics were observed among the isolated pathogens. Access to this current data will help in understanding the local burden of diarrhoeal disease and contribute to better design of prevention programmes.

Key words: Antibiotic, resistance, Bangladesh, bacterial pathogens, diarrhoeal diseases.

INTRODUCTION

Diarrhoeal disease is among the most common causes of morbidity and mortality in developing countries such as Bangladesh. In all age groups severe diarrhoea can lead to hospitalization, serious sequelae such as haemolytic uraemic syndrome, and in some cases death [1]. Although most diarrhoeal episodes are self-limiting, it would be ideal to be able to prevent diarrhoea, especially the more severe episodes which have a higher likelihood of progressing to serious complications. Some prevention strategies such as improved water and sanitation and basic hygiene practices do not require knowledge of diarrhoeal aetiology, but others such as vaccination would benefit greatly from a comprehensive understanding of the overall burden of pathogen-specific diarrhoeal disease [2].

Fluid and electrolyte replacement by oral hydration or intravenous fluid therapy is the treatment of choice for diarrhoeal disease. However, antibiotic therapy is indicated in some circumstances [3]. The progressive increase in antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern. The acute diarrhoeal diseases for which antimicrobial therapy is clearly effective include shigellosis, cholera, and campylobacteriosis.

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However, for campylobacteriosis, the diagnosis is usually too late for antimicrobial therapy to be effective [4]. Of the bacteria causing diarrhoeal disease, *Salmonella* spp. continue to be a major public health problem. Although most *Salmonella* infections are self-limiting, serious sequelae, including systemic infection and death, can occur [5, 6]. Incidence rates and aetiological agents of acute childhood diarrhoeal disease differ between developing and developed countries [7]. Access to current antimicrobial susceptibility data is of importance to clinicians and is of particular significance to physicians treating hospitalized patients [8]. Knowledge about susceptibility patterns of bacteria in different geographical areas is necessary to control bacterial resistance [9].

The aim of our study was to detect diarrhoea-causing bacterial pathogens in stool samples and rectal swabs collected from diarrhoeal patients hospitalized at Dhaka Hospital, ICDDR,B, Dhaka and domiciliary patients of Dhaka city. The intention was to observe the trends in bacterial pathogens associated with diarrhoeal diseases along with current antimicrobial susceptibility patterns of the isolated bacterial pathogens over a 4-year period.

METHODS

From January 2005 to December 2008, a total of 56 132 stool samples and rectal swabs were received from hospitalized (in-patients) and domiciliary (out-patients) diarrhoeal patients at Clinical Laboratory Services of ICDDR,B; Dhaka, Bangladesh. There were 15 965, 13 278, 13 137 and 13 752 samples in 2005, 2006, 2007 and 2008, respectively. It was not possible to exclude repeat samples from the same patient during an episode of diarrhoeal illness. All these samples were tested for the presence of *Shigella*, *Salmonella*, *Vibrio* and *Campylobacter* (when requested) and antimicrobial susceptibility tests were also performed.

Of the stool samples and rectal swabs received, a total of 15 783 samples (2533, 5104, 2299 and 5847 samples in four consecutive years) were tested for the presence of *Campylobacter* spp.

Bacteriological isolation

Collected stool samples and rectal swabs were directly inoculated onto McConkey (MC) agar (Difco, BBL), *Salmonella-Shigella* (SS) agar (Difco, BBL), taurocholate tellurite gelatin agar (TTGA) and *Brucella* agar (Difco, BBL) supplemented with 5% sheep's blood and five antibiotics (amphotericin B, cephalothin, polymyxin B, trimethoprim, vancomycin) for the isolation of *Salmonella*, *Shigella*, *Vibrio* and *Campylobacter* spp. respectively. All the plates were incubated at 37 °C for 18–24 h except for *Brucella* agar, which was incubated at 42 °C in an anaerobic jar with a CampyGen pack (CN0025, Oxoid Ltd, UK) for 48 h. Along with direct streaking, each sample was enriched in selenite broth (Difco, BBL) and bile peptone broth at 37 °C for 18–24 h to enhance the isolation of *Salmonella* spp. and *Vibrio* spp., respectively. The enrichment broth for *Salmonella* was subcultured onto SS agar and the enrichment broth for *Vibrio* was subcultured onto TTGA agar and incubated at 37 °C for 18–24 h. Bacterial enteric pathogens were identified by colony characteristics, and by biochemical tests using conventional and API 20 biochemical profiles (bioMérieux, France) when necessary. Isolates were further confirmed serologically using commercially available specific antisera (Denka Seiken, Japan). *Campylobacter* spp. isolates were differentiated as *C. jejuni* and *C. coli* by the hippurate hydrolysis test.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disc diffusion method on Mueller–Hinton agar (Difco, BBL) following CLSI guidelines [10]. For *Campylobacter* spp. blood agar containing 5% sheep's blood was used. Susceptibility testing was performed for ampicillin (10 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), cotrimoxazole (25 μg), erythromycin (15 μg), nalidixic acid (30 μg) and tetracycline (30 μg). Antibiotic discs were obtained from Oxoid, UK. For *V. cholerae*, interpretive criteria for the zones of inhibition produced by ciprofloxacin and erythromycin discs have not been developed. However, interpretation was based on CLSI criteria for Enterobacteriaceae and multi-laboratory study findings, respectively [11]. Interpretation of antimicrobial susceptibility for *Campylobacter* spp. was done using CASFM guidelines [12]. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Statistical analysis

Trends in isolation as well as antimicrobial susceptibilities of isolated diarrhoeal pathogens were

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Table 1. *Bacterial pathogens isolated from diarrhoeal patients in Bangladesh from January 2005 to December 2008*

Organisms	Year				Total	Percentage (no. of total isolates, $N=14\,428$)
	2005 (4424)*	2006 (3855)	2007 (2977)	2008 (3172)		
Aeromonas spp.	625	459	273	491	1848	12·81
Campylobacter spp.*	198	483	443	631	1755	12·16
C. coli	17	99	94	177	387	2·68
C. jejuni	181	380	349	454	1364	9·45
Plesiomonas shigelloides	195	185	119	147	646	4·48
Salmonella spp.	316	270	163	171	920	6·38
Non-typhoidal *Salmonella* spp.	239	194	118	100	651	4·51
S. Paratyphi	6	4	22	16	48	0·33
S. Typhi	71	72	23	55	221	1·53
Shigella spp.	928	821	497	679	2925	20·27
Sh. boydii	193	159	97	132	581	4·03
Sh. dysenteriae	53	60	55	45	213	1·48
Sh. flexneri	587	475	264	400	1726	11·96
Sh. sonnei	92	109	57	69	327	2·27
Vibrio spp.	2107	1609	1440	1030	6186	42·87
V. cholerae non-O1 non-O139	71	53	42	67	233	1·61
V. cholerae O1 El Tor Inaba	560	855	681	285	2381	16·50
V. cholerae O1 El Tor Ogawa	1465	699	716	673	3553	24·63
V. parahaemolyticus	8	1	0	5	14	0·10
V. cholerae O139	1	1	0	0	2	—

* Values in parentheses are the number of isolates in each year; *Campylobacter* spp. were isolated from 2533, 5104, 2299 and 5847 tested samples in four consecutive years.

determined using χ^2 for trend in Epi Info version 6 software (CDC, USA). A P value $\leqslant 0{\cdot}05$ was considered significant for all comparisons.

RESULTS

During the study period from January 2005 to December 2008, a total of 56 132 stool samples and rectal swabs were received from diarrhoeal patients who ranged in age from 1 day to 80 years with a mean age of 13 years. For these four study years, 27·7%, 29·3%, 22·7% and 23·1% of the tested samples were found to be culture positive ($P<0{\cdot}001$) and overall 25·7% of all the received samples were culture positive. In 2005, the isolates numbered 4424, whereas in 2006, 2007 and 2008, there were 3855, 2977 and 3172 isolations, respectively. Table 1 shows the distribution of individual species. Overall, *Vibrio* spp. were the most predominant microorganisms found to be associated with diarrhoeal diseases in this region. *Shigella* spp. were the second most frequently isolated pathogens. Other frequently isolated pathogens included *Aeromonas* spp., *Salmonella* spp., and *Plesiomonas shigelloides*.

Vibrio spp. isolation decreased to 32·5% of the total isolates in 2008, whereas in 2005, 2006 and 2007 they accounted for 47·6%, 41·7% and 48·4% of the total (χ^2 for linear trend $=110{\cdot}6$, $P<0{\cdot}001$). Among the total *Vibrio* spp. isolated, 96% were identified as *V. cholerae* serogroup O1 El Tor biotype, 3·8% were *V. cholerae* serogroup non-O1 non-O139, and 0·2% were *V. parahaemolyticus*. *V. cholerae* O139 was identified only twice, once in 2005 and again in 2006. The *V. cholerae* O1 El Tor Ogawa serotype predominated throughout most of the study period although from August 2006 to August 2007 the Inaba serotype was more common (Fig. 1).

A small decrease in the incidence of *Shigella* spp. was seen in 2007 but this was not statistically significant. Of the isolated *Shigella* spp., 59% were *S. flexneri*, 19·9% were *S. boydii*, 11·2% were *S. sonnei* and 7% were *S. dysenteriae* (not type 1). There was a decreasing trend in the isolation rate of *Salmonella* spp., they comprised 7·1%, 7·0%, 5·5% and 5·4% of the total isolates in four consecutive years (χ^2 for linear trend $=13{\cdot}8$, $P<0{\cdot}001$). Non-typhoidal *Salmonella* spp. were more frequently isolated than typhoidal *Salmonella* spp. and there was also a decreasing trend

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Table 2. *Percentage of antimicrobial resistance in* Vibrio cholerae *O1 and non-O1 non-O139 isolates from diarrhoeal patients in Bangladesh*

	V. cholerae O1				*V. cholerae* non-O1 non-O139			
Antibiotic	2005 (2025)*	2006 (1554)	2007 (1397)	2008 (958)	2005 (71)	2006 (53)	2007 (42)	2008 (67)
CIP	0	0	0	0	1	0	0	0
E^R	62	34	2	0	6	0	3	0
E^I	7	65	98	100	7	89	93	94
SXT	99	100	98	99	34	30	41	31
TE	73	50	52	70	11	0	17	5

CIP, Ciprofloxacin; E^R, erythromycin resistant; E^I, erythromycin intermediate; SXT, cotrimoxazole; TE, tetracycline.
* Values in parentheses are the number of isolates.

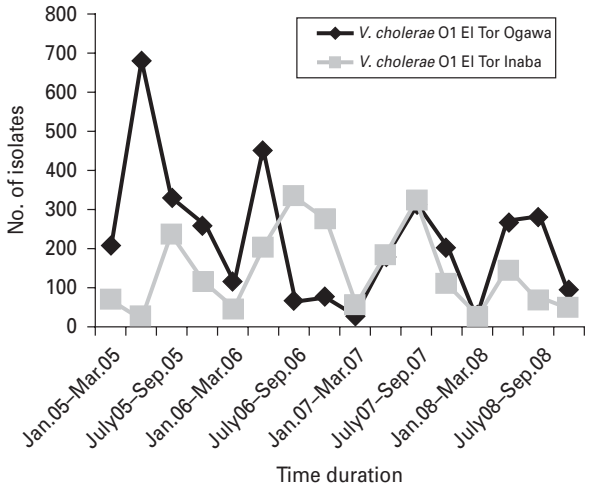

Fig. 1. Distribution of *Vibrio cholerae* O1 El Tor serotypes Ogawa and Inaba during the study period (January 2005 to December 2008).

in their isolation (χ^2 for linear trend = 25·4, $P < 0{\cdot}001$). There was a sharp increase in isolation of *Campylobacter* spp.; 7·8%, 9·5%, 19·3% and 10·8% of tested samples in four consecutive years revealed *Campylobacter* (χ^2 for linear trend = 25·1, $P < 0{\cdot}001$). During the study period, a total of 1755 *Campylobacter* isolates were obtained from the tested samples and of these isolates 77·7% were identified as *C. jejuni* and 22·1% as *C. coli*. In the first 3 years, there was a decreasing trend in isolation of *Aeromonas* spp. being 14·1%, 11·9% and 9·2% of the isolates (χ^2 for linear trend = 41·2, $P < 0{\cdot}001$); however, the isolation rate increased to 15·5% in 2008. There was no significant increase or decrease in the isolation of *Plesiomonas shigelloides*; overall they comprised 4·5% of the total isolates.

Overall, 99% of *V. cholerae* serogroup O1 isolates showed resistance to cotrimoxazole and 61% to tetracycline, but the isolates were susceptible to ciprofloxacin. Reduced susceptibility to erythromycin in serogroup O1 isolates increased significantly between 2005 and 2008 ($P < 0{\cdot}001$). Thirty-four percent of *V. cholerae* non-O1 non-O139 isolates showed resistance to cotrimoxazole, and reduced susceptibility to erythromycin increased from 7% in 2005 to 94% in 2008 (χ^2 for linear trend = 109·3, $P < 0{\cdot}001$). All the isolates were susceptible to ciprofloxacin (Table 2).

Shigella spp. were increasingly resistant to nalidixic acid and ampicillin. Overall, 51%, 83% and 70% of the *S. flexneri* isolates showed resistance to ampicillin, nalidixic acid and cotrimoxazole, respectively and resistance to ciprofloxacin increased from 1% of isolates in 2005 to 34% in 2008 (χ^2 for linear trend = 262, $P < 0{\cdot}001$). Overall, 55%, 84% and 52% of *S. boydii*, *S. sonnei* and *S. dysenteriae* (not type 1), respectively showed resistance to nalidixic acid while cotrimoxazole resistance was 53%, 97%, and 73%, respectively. The overall resistance to ampicillin was below 40% for these isolates (Table 3). Overall, *Salmonella* spp. showed resistance to nalidixic acid (52%), ampicillin (30%), cotrimoxazole (24%) and chloramphenicol (19%); 33% showed reduced susceptibility to ciprofloxacin whereas 3% were completely resistant (Table 4).

Thirty-one percent and 37% of the *Campylobacter* isolates were resistant to ampicillin and tetracycline, while ciprofloxacin resistance increased from 65% in 2005 to 88% in 2008 (χ^2 for linear trend = 39·4, $P < 0{\cdot}001$). However, the isolates were mostly susceptible to erythromycin (Table 5).

DISCUSSION

The present study traces the trends of bacterial pathogens associated with diarrhoeal disease in Dhaka,

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Table 3. *Percentage of antimicrobial resistance in* Shigella *spp. isolates from diarrhoeal patients in Bangladesh*

	S. flexneri				*S. boydii*				*S. sonnei*				*S. dysenteriae*			
Antibiotic	2005 (587)*	2006 (475)	2007 (264)	2008 (400)	2005 (193)	2006 (159)	2007 (97)	2008 (132)	2005 (92)	2006 (109)	2007 (57)	2008 (69)	2005 (53)	2006 (60)	2007 (55)	2008 (45)
AM	34	54	54	61	34	28	46	44	12	6	2	6	26	32	24	44
CIP	1	5	14	34	0	0	0	5	0	0	0	15	0	0	0	0
NA	73	82	88	90	55	62	51	52	80	86	79	90	49	55	56	49
SXT	67	75	69	70	54	43	61	55	98	97	97	97	72	68	76	76

AM, Ampicillin; CIP, ciprofloxacin; NA, nalidixic acid; SXT, cotrimoxazole.
* Values in parentheses are the number of isolates.

Table 4. *Percentage of antimicrobial resistance in* Salmonella *spp. isolates from diarrhoeal patients in Bangladesh*

	Salmonella spp.			
Antibiotic	2005 (318)*	2006 (276)	2007 (189)	2008 (175)
AM	28	30	35	25
C	19	23	16	16
CIP	1	4	4	4
CRO	6	12	8	16
NA	60	50	40	56
SXT	25	27	20	23

AM, Ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; NA, nalidixic acid; SXT, cotrimoxazole.
* Values in parentheses are the number of isolates.

Table 5. *Percentage of antimicrobial resistance in* Campylobacter *spp. isolates from diarrhoeal patients in Bangladesh*

	Campylobacter spp.			
Antibiotic	2005 (198)*	2006 (483)	2007 (443)	2008 (631)
AM	29	29	30	37
CIP	65	84	87	88
E	0	1	2	3
TE	35	35	34	43

AM, Ampicillin; CIP, ciprofloxacin; E, erythromycin; TE, tetracycline.
* Values in parentheses are the number of isolates.

the capital city of Bangladesh, over a 4-year period. In addition, changes in the antimicrobial resistance patterns of the associated bacterial pathogens are presented.

V. cholerae O1, *Shigella* spp., enterotoxigenic *Escherichia coli*, *C. jejuni* and rotaviruses are important diarrhoeal pathogens in Bangladesh [13–19]. In the present study, prepotency of *Vibrio* spp. was observed in four consecutive years with a decreasing trend in isolation in the last year (2008). Among *Vibrio* spp., *V. cholerae* serogroup O1 El Tor biotype was the most predominant. Re-emergence of the Inaba serotype and a sharp decrease in isolation of Ogawa serotype from August 2006 to August 2007 is also indicated by our study. *Shigella* spp. prevailed as the second most isolated organism with a decreased isolation rate in 2007. *S. flexneri* predominated among the isolated *Shigella* spp. followed by *S. boydii*, *S. sonnei* and *S. dysenteriae*; however, no *S. dysenteriae* type 1 was isolated in the study period. This finding is similar to other previous reports from Bangladesh [20] and other developing countries such as Brazil [21], Egypt [22], Indonesia [23], Tanzania [24] and Thailand [25]. There was a decreasing trend in isolation of *Salmonella* spp.; however, for *Campylobacter* spp. the trend was increasing. In the first 3 years of the study *Aeromonas* spp. showed a decreasing trend in isolation but the isolation rate increased in 2008.

During the study period, except for *Campylobacter* spp., there was a decreasing trend in isolation of bacterial pathogens. Although the actual reason for this decreasing trend is not clear, it might be due to an increased awareness in the urban population of infection risks and consequent improvements in hygiene and sanitation practices. On the other hand, the increase in isolation of *Campylobacter* might be due to a change in food habits. In addition, improvements in laboratory techniques and staff practices might have had an indirect influence over the study period.

In the present study, *V. cholerae* isolates were frequently resistant to cotrimoxazole and tetracycline, but sensitive to ciprofloxacin; *Shigella* spp. showed varying degree of resistance to cotrimoxazole,

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nalidixic acid and ampicillin, and a sharp increase in ciprofloxacin resistance was also observed for *S. flexneri* isolates. *Salmonella* spp. showed resistance to nalidixic acid, ampicillin, cotrimoxazole and chloramphenicol, whereas a similar kind of study in Indonesia reported *Shigella* spp. increasingly resistant to ampicillin, cotrimoxazole, chloramphenicol and tetracycline. In an Indonesian study, *Salmonella* spp. were sensitive to all the antibiotics tested and a small number of *V. cholerae* O1 showed resistance to ampicillin, cotrimoxazole, chloramphenicol and tetracycline [23]. A slightly earlier report on two cholera outbreaks in Tanzania (1997 and 1999) showed a similarly high frequency of cotrimoxazole resistance in *V. cholerae* O1 isolates compared to the present study. Increasing resistance to chloramphenicol, ampicillin and tetracycline was also seen in the Tanzanian outbreaks [26]. In another report *Shigella* spp. were found to be 81·8% resistant to ampicillin, 72·7% to chloramphenicol, 96·9% to tetracycline and 87·9% to cotrimoxazole in Tanzania [24]. In Bangladesh, multidrug resistance of *V. cholerae* O1 from urban and rural areas was reported, the strains were resistant to tetracycline, erythromycin cotrimoxazole and furazolidone; reversal of susceptibility to tetracycline of the strains after a 2-year period was also reported [11].

Antibiotic resistance among the *Salmonella* spp. isolated was relatively frequent except for ciprofloxacin, where resistance was rare. In contrast, there was a marked increase in ciprofloxacin resistance in *Campylobacter* spp. between 2005 and 2008. An increase in ciprofloxacin-resistant *Campylobacter* strains has been reported worldwide with rates varying between 45% and 83% [27–29]. A systemic surveillance over an 11-year period in Karachi, Pakistan also reported a steady rise in resistance against ampicillin, tetracycline and ofloxacin in *Campylobacter* isolates [30]. These findings call into question the use of ciprofloxacin as a drug of first choice for empirical treatment of campylobacteriosis. *Campylobacter* isolates resistant to erythromycin were quite rare and this antibiotic may be more useful for the treatment of campylobacteriosis in Dhaka.

The increase in antibiotic resistance observed in this study may be a reflection of the overuse and misuse of antibiotics due to their easy availability over the counter from local drug stores. Recent use of antibiotics in animal husbandry, and fruit and vegetable cultivation might have played some role in the transfer of resistance factors. Nosocomial infection by multidrug-resistant bacteria is common problem in Bangladesh [31, 32], which is also an important cause of the emergence and spread of multidrug-resistant bacteria. To prevent the spread of antibiotic resistance among the diarrhoea-causing bacterial pathogens dispensing of antibiotics without a prescription should be restricted, community-wide education about the responsible use of antibiotics should be promoted, physicians should encourage patients to start antibiotic therapy after culture and sensitivity results have been obtained and patients should complete the full course of antibiotics.

Understanding the burden of pathogen-specific diarrhoeal disease is important for planning effective control programmes and for the overall reduction of diarrhoeal disease in persons of all ages. Current data on the local burden of bacterial pathogens and their susceptibility pattern will help physicians in the empirical treatment of diarrhoeal patients in this endemic area.

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DECLARATION OF INTEREST

None.

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