

Etiological diversity of diarrhoeal disease in Bangladesh

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Abstract

Background: This study compared the diversity of common diarrhoeal pathogens and antimicrobial susceptibility in four hospitals in Bangladesh.

Methodology: A total of 13,959 diarrhoea patients, comprising rural Mirzapur [2,820), rural Matlab (2,865), urban Dhaka (5,287) and urban Mirpur (2,987) were included under the diarrhoeal disease surveillance system of icddr,b during 2010-2011; stool specimens were tested for *Shigella* spp., *Vibrio cholerae*, enterotoxigenic *Escherichia coli* and rotavirus.

Results: Rotavirus was highest in Mirzapur (28%) followed by Dhaka (24%), Matlab (19%) and Mirpur (18%). Overall, *Shigella* was significantly more prevalent in rural sites (Mirzapur 13% and Matlab 7%), than in urban sites (Dhaka 3% and Mirpur 3%). *Vibrio cholerae* was more common in the urban sites of Dhaka (14%) and Mirpur (12%). 72% of *Shigella* isolates were susceptible to ciprofloxacin in Mirzapur, and 88% to mecillinam. In Dhaka, the figures for *Shigella* were 65% and 50%, in Matlab 65% and 85%, and in Mirpur 59% and 92% respectively. Susceptibility of *Shigella* to azithromycin and ceftriaxone in Dhaka was 74% and 95%, and in Mirpur 88% and 92% respectively. *Vibrio cholerae* showed the highest resistance to trimethoprim-sulfamethoxazole (100% in Mirpur) and lowest resistance to ciprofloxacin (0% in Dhaka, Matlab and Mirpur) and azithromycin (30% in Dhaka to 7% in Mirzapur). Multidrug resistance (≥ 3 antibiotics) for *Shigella* were: Mirzapur (50%); Dhaka (36%); Matlab (23%) and Mirpur (37%); and for *V. cholerae* it was 26%, 37%, 49% and 23% respectively.

Conclusion: The isolation rates and antimicrobial susceptibility of *Shigella* spp. and *V. cholerae* along with rotavirus differed significantly in certain geographical sites.

Key words: antimicrobial resistance; diarrhoea; etiology; rural; susceptible; urban

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Introduction

Diarrhoeal disease is a leading public health concern in resource-poor countries. It is the second biggest killer of children under age five, claiming the lives of approximately 1.4–1.8 million children globally [1,2]. The average diarrhoeal disease incidence in such settings is 3.2 episodes per child per year [3], though this can be as high as 12 episodes [4]. The majority of morbidity and mortality is observed in children under two years old [5]. Moreover, diarrhoea has been found to have significant long-term impacts; it negatively affects nutritional status, which increases susceptibility to other infectious diseases [6], and has adverse effects on physical and mental development which has been linked to reduced productivity during adulthood [7].

Together, enterotoxigenic *Escherichia coli* (ETEC), rotavirus, *Vibrio cholerae* and *Shigella* spp.

are the major fatal etiologic diarrhoea-causing organisms in children in developing countries [8,11]. Although diarrhoea-associated mortality has roughly halved in the past 20 years, there is no indication that the disease morbidity has decreased [12]. Additionally, and as with other diseases, there has been an alarming increase in the rate of resistance to commonly-used antimicrobials.

The spectrum of pathogens, their relative geographical spread, and level of interaction with different populations and health systems is an important consideration for antimicrobial resistance screening. The geographic distribution of pathogens is particularly important as pathogen populations are often spatially structured by location. Several studies have examined geographic heterogeneity of organisms. One such study focused on the diversity of *Shigella flexneri* strains isolated in Egypt and their

relative antimicrobial susceptibility [13]. Another study examined regional variation in rotavirus detection rates from 1984 to 2006 [14] and diversity in molecular epidemiology of rotavirus was also shown between central and southeastern Europe [15,16]. However, there is limited information on variation of enteric pathogens and their susceptibility patterns in Bangladesh. Thus, this study aimed to determine the effect of geographical diversity on distribution and antimicrobial susceptibility patterns of common enteric pathogens in stool specimens of diarrhoeal patients between four different diarrhoeal disease surveillance sites.

Methodology

Study sites

Kumudinini Hospital - Mirzapur

Kumudini Hospital, established in 1938 in Mirzapur (Tangail) and located 60 kilometers northwest of Dhaka is a philanthropic institution providing health services to the surrounding poor rural population. Since 1982, they have started a separate inpatient and outpatient diarrhoeal treatment unit composed of 20 beds. Nearly 1500 diarrhoeal patients report to this facility each year for treatment. The diarrhoeal disease surveillance system operates around the clock to obtain information and fecal specimens from the residents of the regional Demographic Surveillance System area who report with diarrhoeal illnesses regardless of disease severity.

Dhaka Hospital - Dhaka

Free treatment is offered by the icddr,b to at least 140,000 people a year and has operated a diarrhoeal disease surveillance system (DDSS) since 1979. The DDSS currently collects information on clinical, epidemiological and demographic characteristics, feeding practices (particularly of infants and young children), and the use of drug and fluid therapy at home of every 50th patient, irrespective of age, sex, disease severity or socioeconomic status by distributing a structured questionnaire. The generated data provides valuable information to hospital clinicians in their decision-making processes and enables the detection of emerging pathogens and early identification of outbreaks and their locations.

Matlab Hospital-Matlab

Since 1963, icddr,b has been maintaining a treatment facility in rural Matlab for treating diarrhoeal patients. It provides free treatment to 12,000 to 20,000 diarrhoeal patients reporting from the

Health and Demographic Surveillance System (HDSS) area and other adjoining sub-districts. The DDSS in Matlab Hospital began in the year 2000 and enrolls all diarrhoeal patients coming from the HDSS irrespective of age, sex and socioeconomic status.

Mirpur Treatment Centre-Mirpur

This 60-bed urban hospital at Mirpur began operations in April 2009. More than 12,000 patients receive annual care at this facility. Every 10th patient to attend the facility is included into the icddr,b DDSS irrespective of age, sex, socioeconomic status and geographical location.

Study design and admittance of patients

The present diarrhoeal disease surveillance study was conducted in the four different hospitals in Bangladesh outlined above. Cases were defined as individuals with diarrhoea, seeking medical care at one of the health facilities described above, either as an inpatient or an outpatient. Diarrhoea was defined as the passage of at least three loose or watery stools within a 24-hour period and dysentery was defined as the presence of blood in any stool. Dehydration was defined as moderate or severe dehydration. Some dehydration was recorded if two or more of the following symptoms were observed: restlessness, irritability; sunken eyes; drinks eagerly, thirsty; and skin pinch goes back slowly [17]. Severe dehydration was noted if two or more of the following symptoms were observed: lethargy or unconsciousness; sunken eyes; drinks poorly, or not able to drink; and skin pinch goes back very slowly (≥ 2 seconds) [17]. Patients were admitted around the clock without considering the duration of their episode, prior use of antimicrobials or whether they were part of an outbreak.

Optimal patient care was ensured in all the facilities that included proper rehydration either by oral saline or intravenous fluid with appropriate and rationale use of antimicrobials. Additionally, proper counselling about personal hygiene and dietary practices, especially continuation of breastfeeding, were provided by a trained research assistant. Trained physicians and nurses were responsible to ensure optimal patient care including assessment of dehydration as well as electrolyte imbalance.

Sample size estimation

Assuming an isolation rate of any pathogen as low as ~10% ($p=0.10$, $q=0.90$, $Z=1.96$, and $d=0.04$) the desired sample size was estimated to be approximately 216 positive cases. To obtain that number of any specific pathogen, 2160 stool specimens had to be examined.

Specimen collection and laboratory procedure

All patients who provided informed consent and came to the Kumudini Hospital in Mirzapur with diarrhoea provided a single fresh, whole stool specimen (at least 3 ml or g). A faecal swab was then placed in Cary-Blair medium in a plastic screw top test tube. Using a Styrofoam container with cold packs, the specimen was transported to the central laboratory in Dhaka within 6 to 18 hours of collection. All patients coming from the HDSS area in Matlab were included and their stool specimens were processed in the Matlab Microbiology Laboratory; a 2% sub sample from Dhaka Hospital and 10% of samples from the Mirpur Treatment Centre were examined in the central laboratory of icddr,b in Dhaka. Each specimen was aliquoted into three serial containers and submitted to the respective laboratories for routine screening of common enteric pathogens such as ETEC [18], *V. cholerae* [19], *Shigella* spp. [19], and rotavirus [20] applying standard methods. Bacterial isolates were tested for susceptibility to antimicrobials by the disk-diffusion test.

For ETEC, stool samples were plated onto MacConkey agar, and the plates were incubated at 37°C for 18 h. Six lactose-fermenting individual colonies morphologically resembling *E. coli* were tested [21]. For *V. cholerae*, stool samples were plated on taurocholate-tellurite-gelatin agar [22] and gelatin agar (Difco, Detroit, USA); after overnight incubation of plates, serological confirmation of suspected *Vibrio* spp. colonies was carried out by slide agglutination [23,24]. *Shigella* spp. were isolated and identified in the enteric microbiology laboratory using standard biochemical and microbiological methods [25]. Stool specimens were streaked onto MacConkey and *Shigella-Salmonella* agar plates and incubated overnight at 37°C. Non-lactose fermenting colonies characteristically resembling *Shigella* were inoculated into a Kligler's iron agar tube for typical reaction, mannitol fermentation, citrate utilization, urease and indole production, and lysine decarboxylation. *Shigella* serotypes were confirmed by slide agglutination with polyvalent somatic (O) antigen grouping sera, followed by testing with monovalent

antisera for specific serotype identification (Denka Seiken, Tokyo, Japan). In cases where no agglutination occurred with bacterial colonies, the test was repeated with boiled suspensions of bacteria. *S. flexneri* isolates that were not typeable with commercial antisera were typed using a panel of monoclonal antibodies specific for *S. flexneri* group and type factor antigen [26].

Group A rotavirus-specific VP6 antigen was detected in the stool specimens using solid phase sandwich-type enzyme immunoassay modeled according to the commercial kit [UTF-8 ProSpecT Rotavirus Microplate Assay (Oxoid Ltd, Basingstoke, UK). Positive and negative controls were included in the first of every test. Quality control of the EIA test was routinely done using rotavirus positive samples with known OD values [27].

Bacterial susceptibility to antimicrobial agents was determined by the disk diffusion method as recommended by the Clinical Laboratory Standards Institute (CLSI 2010, June update) [28] with commercial antimicrobial discs (Oxoid Ltd, Basingstoke, UK). The antibiotic discs used in this study were ampicillin (10 µg), tetracycline (30 µg), mecillinam (25 µg), nalidixic acid (30 µg), trimethoprim-sulfamethoxazole [cotrimoxazole (TMP-STX)], (25 µg), and ciprofloxacin (5 µg) for *Shigella*. For *V. cholerae*, tetracycline (30 µg), trimethoprim-sulfamethoxazole [cotrimoxazole (TMP-STX)] (25 µg), erythromycin (15 µg), ciprofloxacin (5 µg), and azithromycin (15 µg) antibiotic discs were used. For ETEC susceptibility to azithromycin (15 µg), ciprofloxacin (5 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole [cotrimoxazole (TMP-STX)] (25 µg), tetracycline (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), doxycycline (30 µg), mecillinam (25 µg), norfloxacin (10 µg), nalidixic acid (30 µg) and streptomycin (10 µg) were tested. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as control strains for susceptibility studies (28).

Data management and analysis

The Statistical Package for Social Sciences (SPSS) Windows (Version 15.2; Chicago, USA) and Epi Info (Version 6.0, USD, Stone Mountain, USA) were used for data entry and analysis included descriptive methods. Inter site differences in the proportions were compared using a Chi-square test; a probability of <0.05 was considered statistically significant.

Ethical consideration

This study was approved by the Research Review Committee and Ethical Review Committee of icddr,b. All individuals were admitted and their stool specimens were collected for microbiological assessment once they provided informed consent. For infants and children aged less than 10 years, informed consent was given by their parents or caregivers.

Results

Significant difference in socio-demographic characteristics, sanitation practices and clinical characteristics of the diarrhoeal patients were observed at the different sites (Table 1). The proportion of male patients attending health facilities was higher in Dhaka and Mirzapur. Maternal illiteracy rate was comparatively higher in urban sites. However, lower socio-economic status was observed among rural sites (Matlab and Mirzapur). The reported use of sanitary latrines was lower between rural Matlab and urban Mirpur, whereas the reported use of antimicrobials prior to hospital admittance was significantly higher in Mirzapur compared to other sites. The proportion of some or severe dehydration cases with watery stool was significantly higher in Dhaka and Mirpur. Conversely, a higher number of patients arrived with abdominal pain and a fever for both rural sites compared to the urban sites.

Overall, rotavirus was the most commonly isolated organism across all sites. In Mirzapur, it accounted for 28% of cases, in Dhaka 24%, 19% in Matlab and 18% in Mirpur. *Shigella* was the second most prevalent organism in Mirzapur (13%) whereas it was the least commonly detected organism in Dhaka (3%), Matlab (7%) and Mirpur (3%). In these three sites, *V. cholerae* was the second most prevalent organism (14%, 9%, 12% respectively). In Dhaka and Mirzapur, ETEC the third most common organism (8 and 3%, respectively); no attempt was made to identify ETEC in samples from Matlab and Mirpur.

Among children less than 5 years old, rotavirus was even more commonly detected across the sites (Table 2). The isolation rate for *Shigella* among children aged less than 5 years was highest in Mirzapur, compared to other three sites. Variation was noted in the dominant circulating *Shigella* spp. in each location. *S. flexneri* was the most prevalent *Shigella* serotype in all facilities followed by *S. sonnei* than *S. boydii* and *S. dysenteriae* (Table 2).

Nearly 72% of *Shigella* isolates in Mirzapur were susceptible to ciprofloxacin, while 88% were susceptible to mecillinam – both of which are often used for treating shigellosis in Bangladesh (Table 3). Twelve to fifty nine percent of the isolates were susceptible to other antibiotics such as ampicillin, TMP-STX, and nalidixic acid (Table 3). In Dhaka, 50% and 65% of *Shigella* isolates were susceptible to ciprofloxacin and mecillinam respectively. In Matlab, 65% and 85% of *Shigella* isolates were susceptible to ciprofloxacin and mecillinam, while in Mirpur, the figures stand at 59% and 82% (Table 3). *Shigella* susceptibility to ampicillin ranged from 45% (Matlab) to 73% (Mirpur); TMP-STX ranged from 28% (Mirzapur and Dhaka) to 35% (Mirpur) and nalidixic acid from 1% (Dhaka) to 20% (Matlab). In Dhaka and Mirpur, susceptibility to azithromycin stood for 74% and 88%, and ceftriaxone 95% and 92% respectively (Table 3).

V. cholerae exhibited much greater geographic heterogeneity in terms of resistance patterns to antimicrobials than *Shigella* serotypes. Susceptibility testing for azithromycin was conducted in all sites except Matlab, and susceptibility ranged from 93% in Mirzapur to 70% in Dhaka and 83% Mirpur (Table 4). Ciprofloxacin was the drug with the least detected resistance. TMP-STX showed the greatest variation in resistance, with 2% of strains susceptible in Dhaka, 10% in Mirzapur, 18% in Matlab and none of the isolates were susceptible in Mirpur. Recorded resistance to erythromycin was also high (100% in Mirpur) (Table 4).

Although Mirzapur and Dhaka both isolated ETEC, only Mirzapur characterized the antibiotic resistance associated with these isolates; 97% of the ETEC isolates were susceptible to mecillinam followed by ciprofloxacin (80%), norfloxacin (80%), streptomycin (74%), ceftriaxone (66%), TMP-STX (61%), azithromycin (48%), tetracycline (49%), doxycycline (46%), nalidixic acid (20%), ampicillin (20%) and none of the isolates were susceptible to erythromycin in Mirzapur.

Table 1. Characteristics of patients in four diarrhoeal treatment facilities in Bangladesh

Characteristic	Mirzapur; n=2820 (%)	Dhaka; n=5287 (%)	p-value	Matlab; n=2865 (%)	p-value	Mirpur; n =2987 (%)	p-value
Male	1641 (58)	3177 (60)	0.102 ^a	1477 (52)	<0.001 ^a / <0.001 ^b	1649 (55)	0.023 ^a / 0.005 ^c
Illiterate mother	271 (10)	2489 (47)	<0.001 ^a	1043 (36)	<0.001 ^a / <0.001 ^b	1548 (52)	<0.001 ^a / <0.001 ^b / <0.001 ^c
Poor socio-economic status (median monthly family income < US\$100)	1164 (41)	1911 (36)	<0.001 ^a	1565 (55)	<0.001 ^a / <0.001 ^b	1434 (48)	<0.001 ^a / <0.001 ^b / <0.001 ^c
Use of sanitary toilet	2635 (93)	4498 (86)	<0.001 ^a	360 (13)	<0.001 ^a / <0.001 ^b	1687 (57)	<0.001 ^a / <0.001 ^b / <0.001 ^c
Use of antimicrobial prior to hospital visit	2375 (84)	1610 (30)	<0.001 ^a	346 (19)	<0.001 ^a / <0.001 ^b	1069 (48)	<0.001 ^a / <0.001 ^b / <0.001 ^c
Watery stool (lack of mucus/blood)	2038 (72)	5166 (98)	<0.001 ^a	2383 (83)	<0.001 ^a / <0.001 ^b	2939 (99)	<0.001 ^a / <0.001 ^b / <0.001 ^c
Duration of diarrhoea (<1 day)	746 (27)	2382 (45)	<0.001 ^a	1636 (57)	<0.001 ^a / <0.001 ^b	1659 (56)	<0.001 ^a / <0.001 ^b / 0.238 ^c
Dehydration (some or severe)	493 (18)	3181 (61)	<0.001 ^a	1128 (39)	<0.001 ^a / <0.001 ^b	1977 (66)	<0.001 ^a / <0.001 ^b / <0.001 ^c
History of abdominal pain	2007 (71)	1826 (35)	<0.001 ^a	1616 (57)	<0.001 ^a / <0.001 ^b	1243 (42)	<0.001 ^a / <0.001 ^b / <0.001 ^c
Fever (temperature ≥37.8°C)	615 (22)	245 (5)	<0.001 ^a	429 (15)	<0.001 ^a / <0.001 ^b	31 (1)	<0.001 ^a / <0.001 ^b / <0.001 ^c
Death	3 (<1)	13 (0.2)	0.277 ^a	1 (<1)	0.370 ^a / 0.026 ^b	2 (<1)	0.678 ^a / 0.116 ^b / 1.00 ^c

Table 2. Isolation of pathogens among under-five children from four diarrhoeal treatment facilities, Bangladesh, 2010-2011

Pathogen	Mirzapur; n=2321 (%)	Dhaka; n=2641 (%)	p-value	Matlab; n=1422 (%)	p-value	Mirpur; n=1251 (%)	p-value
Rotavirus	756 (33)	1122 (43)	<0.001 ^a	491 (35)	0.231 ^a / <0.001 ^b	443 (35)	0.093 ^a / <0.001 ^b / 0.662 ^c
<i>Shigella</i>	328 (14)	77 (3)	<0.001 ^a	93 (7)	<0.001 ^a / <0.001 ^b	36 (3)	<0.001 ^a / <0.001 ^b / <0.001 ^c
<i>S. flexneri</i>	177 (8)	33 (1)	<0.001 ^a	72 (5)	0.002 ^a / <0.001 ^b	13 (1)	<0.001 ^a / <0.001 ^b / <0.001 ^c
<i>S. sonnei</i>	90 (4)	27 (1)	<0.001 ^a	12 (1)	<0.001 ^a / 0.698 ^b	14 (1)	<0.001 ^a / 0.913 ^b / 0.598 ^c
<i>S. boydii</i>	46 (2)	12 (1)	<0.001 ^a	5 (<1)	<0.001 ^a / 0.818 ^b	8 (1)	0.002 ^a / 0.607 ^b / 0.430 ^c
<i>S. dysenteriae</i>	13 (1)	5 (<1)	0.053 ^a	4 (<1)	0.326 ^a / 0.728 ^b	1 (<1)	0.026 ^a / 0.671 ^b / 0.379 ^c
<i>V. cholerae</i>	46 (2)	135 (5)	<0.001 ^a	38 (3)	0.203 ^a / <0.001 ^b	56 (3)	<0.001 ^a / 0.436 ^b / 0.015 ^c
ETEC	76 (3)	205 (8)	<0.001 ^a	ND	-	ND	-

^a Comparison between Mirzapur with other three sites^b Comparison between Dhaka with Matlab and Mirpur sites^c Comparison between Matlab with Mirpur sites

ND=not done

Table 3. Susceptibility pattern of *Shigella* isolated in different health facilities, Bangladesh, 2010-2011

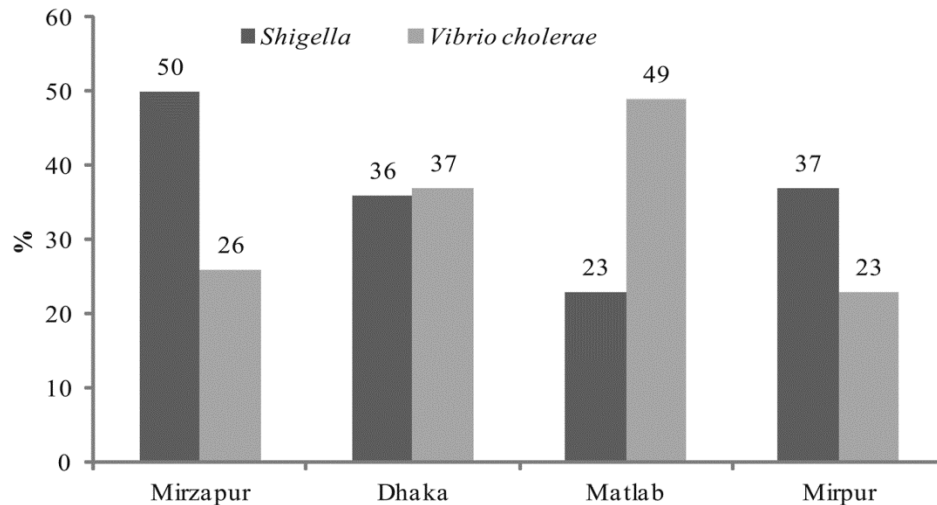
	Mirzapur			Dhaka				p-value	Matlab				p-value	Mirpur			
	Overall n=308 (%)	Absent, n=208 (%)	Present, n=100 (%)	Overall n=98 (%)	Absent, n=44 (%)	Present, n=54 (%)	Overall n=126 (%)		Absent, n=92 (%)	Present, n=34 (%)	Overall n=51 (%)	Absent, n=30 (%)		Present, n=21 (%)	p-value		
Ampicillin	182 (59)	127 (61)	55 (55)	51 (52)	24 (55)	27 (50)	0.129 ^a	57 (45)	41 (45)	16 (47)	0.002 ^a /0.381 ^b	37 (73)	21 (70)	16 (76)	0.168 ^a /0.025 ^b /0.001 ^c		
TMP-STX	85 (28)	53 (26)	32 (32)	27 (28)	13 (30)	14 (26)	0.903 ^a	42 (33)	29 (32)	13 (38)	0.281 ^a /0.433 ^b	18 (35)	11 (37)	7 (33)	0.337 ^a /0.430 ^b /0.940 ^c		
Nalidixic Acid	37 (12)	25 (12)	12 (12)	1 (1)	1 (2)	0	0.002 ^a	25 (20)	21 (23)	4 (12)	0.049 ^a / 0.001 ^b	3 (6)	0	0	0.294 ^a /0.116 ^b /0.037 ^c		
Mecillinam	182 (88)	182 (88)	82 (82)	64 (65)	29 (66)	35 (65)	0.328 ^a	107 (85)	77 (84)	30 (88)	<0.001 ^a /0.001 ^b	42 (82)	25 (83)	17 (81)	0.002 ^a /0.046 ^b /0.844 ^c		
Ciprofloxacin	221 (72)	153 (74)	68 (68)	49 (50)	25 (57)	24 (44)	<0.001 ^a	82 (65)	65 (71)	17 (50)	0.207 ^a /0.032 ^b	30 (59)	20 (67)	10 (48)	0.089 ^a /0.394 ^b /0.541 ^c		
Azithromycin	ND	ND	ND	72 (74)	31 (71)	41 (76)	-	ND	ND	ND	-	45 (88)	25 (83)	20 (95)	0.061 ^b		
Ceftriaxone	ND	ND	ND	93 (95)	41 (93)	52 (96)	-	ND	ND	ND	-	47 (92)	27 (90)	20 (95)	0.492 ^b		

Table 4. Susceptibility pattern of *Vibrio cholerae* isolated in different health facilities, Bangladesh, 2010-2011

	Mirzapur			Dhaka				p-value	Matlab				p-value	Mirpur			
	Overall n=72 (%)	Absent n=33 (%)	Present n=39 (%)	Overall n=448 (%)	Absent, n=294 (%)	Present, n=154 (%)	Overall n=159 (%)		Absent, n=146 (%)	Present, n=13 (%)	Overall n=262 (%)	Absent, n=168 (%)		Present, n=94 (%)	p-value		
Tetracycline	40 (56)	19 (58)	21 (54)	287 (64)	193 (66)	94 (61)	0.209 ^a	124 (78)	115 (79)	9 (69)	<0.001 ^a /0.001 ^b	167 (64)	112 (67)	55 (59)	0.258 ^a /0.955 ^b /0.003 ^c		
TMP-STX	7 (10)	4 (12)	3 (8)	8 (2)	6 (2)	2 (1)	0.001 ^a	29 (18)	25 (17)	4 (31)	0.145 ^a / <0.001 ^b	0	0	0	<0.001 ^a /0.029 ^b / <0.001 ^c		
Erythromycin	31 (43)	14 (42)	17 (44)	1 (0.2)	0	1 (1)	<0.001 ^a	2 (1)	4	0	<0.001 ^a /0.169 ^b	0		0	<0.001 ^a /1.00 ^b /0.142 ^c		
Ciprofloxacin	68 (94)	29 (87)	39 (100)	446 (100)	293 (100)	153 (99)	0.004 ^a	159 (100)	146 (100)	13 (100)	0.008 ^a /1.00 ^b	262 (100)	168 (100)	94 (100)	0.002 ^a /0.533 ^b		
Azithromycin	67 (93)	30 (90)	37 (95)	313 (70)	200 (68)	113 (73)	<0.001 ^a	ND	ND	ND		220 (84)	139 (83)	81 (86)	0.076 ^a / <0.001 ^b		

^a Comparison between Mirzapur with other three sties^b Comparison between Dhaka with Matlab and Mirpur sties^c Comparison between Matlab with Mirpur stie

ND=not done; Absent, antimicrobial use before coming to hospital denied; Present, declaration of antimicrobial use before coming to hospital; TMP-STX, trimethoprim-sulfamethoxazole

Figure: Multidrug resistant pattern of *Shigella* and *Vibrio cholerae* at different sites

Dhaka had the highest average rate of antimicrobial resistance among the different pathogens isolated in each of the study sites. The proportions of multidrug resistance (resistant to 3 or more drugs) for *Shigella* were as followed: Mirzapur (50%); Dhaka (36%); Matlab (23%) and Mirpur (37%). For *V. cholerae* it was 26%, 37%, 49% and 23% respectively (Figure). Antimicrobial use prior to the hospital visit had no significant effect on antimicrobial susceptibility of *Shigella* (Table 3) and *V. cholerae* (Table 4) across the sites with the exception of ciprofloxacin use in Mirzapur and azithromycin use in Mirpur for treatment of *V. cholerae* infection.

Discussion

Large variations in the isolation rate of enteric pathogens were detected across the different sites. *Shigella* spp. infection for example, accounted for 13% of infections in Mirzapur and only 3% in Dhaka and Mirpur, whilst *V. cholerae* was isolated from 14% of samples in Dhaka and 3% in Mirzapur. A number of factors could account for the variations. Such factors include urban-rural differentials in environmental and water-sanitation conditions, host characteristics including age of patient population, baseline nutritional status, immunological characteristics including vaccination rates, disease seasonality, and health care seeking behaviour and prescription practices.

Rotavirus infection usually provokes vomiting followed by diarrhoea in children in their first few years of their lives. By age 5, most children are

expected to have encountered rotavirus. Of rotavirus-associated deaths, nearly 80% of these children die in developing countries owing to poor access to rehydration therapy and a higher prevalence of baseline malnutrition among them [29]. Results from this study indicated that rotavirus is the most prevalent diarrhoea-causing pathogen for both children under 5 and the overall population. Despite the average high burden, significant divergence in isolation rates between the different sites. These findings reinforce the findings from a previous study in Bangladesh (2004) which identified differing trends in incidence rates over a nine year period between Dhaka and Matlab [30] and other studies which have noted geographical variations [14,16].

The burden of shigellosis is greatest in resource-poor countries where it has been estimated to cause as many as 167 million episodes of diarrhoea and more than a million deaths each year [31]. Results from this study indicate that *Shigella* is also prominent in Bangladesh especially among children less than 5 years old. *Shigella* was the second most isolated organism in Mirzapur, but was significantly lower in the other sites, especially Mirpur. A 2011 study conducted with 83,073 patients in Dhaka, revealed that the proportion of hospitalized patients with *Shigella* decreased steadily from 8% to 12% in 1980 to 3% in 2008 [32]. Our results also indicate that *S. flexneri* is the dominant circulating species; this finding supports reports previously indicating the dominance of *S. flexneri* in Bangladesh [32]. The results from the study also illustrate that *V. cholerae* continues to be a major

public health concern in Bangladesh [33, 34, 35]. *V. cholerae* was the primary pathogen in four major flood-associated diarrhoeal epidemics that occurred in Dhaka, Bangladesh, in 1988, 1998, 2004, and 2007 [36]. Despite its prominence, variable isolation rates of *V. cholerae* were documented at the different surveillance sites, with Dhaka having the highest, where cholera accounted for 14% of diarrhoea cases. The results of a study conducted in rural Bangladesh also found much variation among isolation rate of *V. cholerae* [35].

The importance of ETEC in developing countries is under-recognized. ETEC are the most commonly detected bacterial enteropathogens in children less than 5 years old in developing countries [21]. Surveillance of hospitalized cases in Bangladesh indicates that ETEC diarrhoea is also common in individuals over 10 years of age [21]. ETEC isolation was only conducted in Dhaka and Mirzapur, of which Dhaka had a significantly higher rate. In developing countries, ingestion of contaminated weaning food is a known cause of ETEC diarrhoea in infants. Moreover, contaminated food and water sources also contribute to seasonal outbreaks [11]. In urban settings in Bangladesh, ETEC has been found to be present in water sources such as lakes, rivers, and piped water supply systems (drinking water sources) [37] which might explain the higher prevalence rate in Dhaka.

When considering how to best control the spread of diarrhoea, prevention is considered the first priority [1]. Indeed, improving sanitation has been shown to be the most effective way of reducing diarrhoea, followed by adequate hygiene practices and improvement in drinking water [38]. At a treatment level however, when time and resource constraints limit the possibility of running susceptibility testing, it is important to be aware of dominant resistance patterns. For *Shigella*, the major cause of dysentery, the results demonstrate much variation in antimicrobial susceptibility in Bangladesh. Little variation in susceptibility to ampicillin and ciprofloxacin was detected across sites, whereas large variations were detected for TMP-STX, mecillinam and nalidixic acid. Mecillinam is the current drug of choice for treating shigellosis in children aged less than 5 years old, and it demonstrates the lowest detected levels of antimicrobial resistance. However, other antimicrobials, such as azithromycin and ceftriaxone, which are not very commonly used, demonstrated divergent resistant patterns in the two sites where they

were tested (Dhaka and Mirpur). Susceptibility testing for ETEC was only conducted in Mirzapur, where mecillinam was found to be the most effective drug. No conclusions about the heterogeneity of ETEC resistance patterns can be drawn from this data.

The resistance patterns of *V. cholerae* to azithromycin and ciprofloxacin did not exhibit any wide geographic heterogeneity. Several drugs can be used in the treatment of cholera; the most common in Bangladesh is azithromycin. Azithromycin-susceptibility testing was conducted at all sites, except Matlab, and only 5% of *V. cholerae* isolates from Mirzapur were resistant to the most commonly used treatment option. However, susceptibility to other antimicrobials with large detected variation suggests that resistance mechanisms may develop and spread quickly in case of *V. cholerae*. Irrational use of antimicrobials and poor prescription practices are the most frequently quoted factors in driving the current upward trend observed in antimicrobial resistance patterns [39].

The present study also explored the resistance pattern among individuals who took antimicrobials prior to attending the hospital. Significant differences were only observed for ciprofloxacin use in Mirzapur and azithromycin use in Mirpur for treatment of *V. cholerae* infection. However, no statistical differences were found for *Shigella* spp. and none of the antimicrobials impacted treatment outcome. The small sample size, combined with the inability to identify the type of the antimicrobials used at home, were the main underlying circumstances.

Results from potential socio-economic determinant analysis were inconclusive. We were unable to detect any predictors of geographical diversity in isolation rates of enteric pathogens. However, systematic admittance of patients, irrespective of age, sex and disease severity, with large sample size might prove the unique geographical variation of isolation rate of common diarrhoeal pathogens.

The advent of oral rehydration therapy in combination with strides in clean water provision and sanitation have led to impressive reduction in the number of deaths due to diarrhoeal disease [1,3]. The figure has fallen from 4.6 million deaths among children in 1982 to an estimated 2.5 million deaths in 2003 [40,42] and reached 1.3 million in 2008 [43]. Despite these achievements, much remains to be done.

Meeting the Millennium Development Goals target for sanitation (MDG 7) should remain the highest priority (the corresponding water provision target has already been met) [44]. This is particularly pertinent for Bangladesh where 18 million people still practice open defecation [1]. For those who do fall ill with diarrhoea; however, understanding the distribution of local geographical related pathogens is essential when treating them.

Results from hospital-based surveillance of diarrhoeal patients may not be possible to generalise. However, large sample size with unbiased enrollment in four different geographical locations, utilizing high quality laboratory services were the strengths of the present study. The present analysis considered only four common pathogens which were responsible for diarrhoea irrespective of age, sex and geographical diversity. Meanwhile, the study did not collect information about the transmission of pathogens across the different sites such as vector borne transmission that might also play role in disease endemicity.

The results indicate that prevalence and resistance patterns may be geographically distributed. This information can be used by policy makers, and in driving new public health research.

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