

## Aetiologies of diarrhoea in adults from urban and rural treatment facilities in Bangladesh

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### SUMMARY

The objective of our analysis was to describe the aetiology, clinical features, and socio-demographic background of adults with diarrhoea attending different urban and rural diarrhoeal disease hospitals in Bangladesh. Between January 2010 and December 2011, a total of 5054 adult diarrhoeal patients aged  $\geq 20$  years were enrolled into the Diarrhoeal Disease Surveillance Systems at four different hospitals (two rural and two urban) of Bangladesh. Middle-aged [adjusted odds ratio (aOR) 0.28, 95% confidence interval (CI) 0.23–0.35,  $P < 0.001$ ] and elderly (aOR 0.15, 95% CI 0.11–0.20,  $P < 0.001$ ) patients were more likely to present to rural diarrhoeal disease facilities than urban ones. *Vibrio cholerae* was the most commonly isolated pathogen (16%) of the four pathogens tested followed by rotavirus (5%), enterotoxigenic *Escherichia coli* (ETEC) (4%), and *Shigella* (4%). Of these pathogens, *V. cholerae* (19% vs. 11%,  $P < 0.001$ ), ETEC (9% vs. 4%,  $P < 0.001$ ), and rotavirus (5% vs. 3%,  $P = 0.013$ ) were more commonly detected from patients presenting to urban hospitals than rural hospitals, but *Shigella* was more frequently isolated from patients presenting to rural hospitals than urban hospitals (7% vs. 2%,  $P < 0.001$ ). The isolation rate of *Shigella* was higher in the elderly than in younger adults (8% vs. 3%,  $P < 0.001$ ). Some or severe dehydration was higher in urban adults than rural adults ( $P < 0.001$ ). Our findings indicate that despite economic and other progress made, conditions facilitating transmission of *V. cholerae* and *Shigella* prevail in adults with diarrhoea in Bangladesh and further efforts are needed to control these infections.

**Key words:** Adult, diarrhoea, elderly, middle aged, rural, urban.

### INTRODUCTION

Diarrhoeal diseases remain a major public health problem, globally [1]. The incidence of diarrhoea has not declined significantly; however, deaths have been

reduced due to better management, including the use of oral rehydration solutions, zinc, and antimicrobials for treatment of specific aetiological diarrhoeas such as cholera and shigellosis [1–7]. Although young children are the main group affected by diarrhoeal diseases, it is also a problem for adults [8, 9]. Diarrhoea is endemic in most countries and outbreaks and epidemics are common, the latter mostly occurring in developing countries – in both urban and rural areas [10].

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In developing countries, most diarrhoea episodes are caused by infection of the human intestine with enteric pathogens such as bacteria, virus, and parasites [11–13]. Transmission is mostly via the faecal–oral route, and water and food are the main vehicles for transmission, which is facilitated by inadequate knowledge, and poor hygiene practices and sanitary conditions [10, 11]. Individuals with altered immune status, such as neonates and infants, the severely malnourished and the elderly are often at higher risk of diarrhoea [2, 5]. In developing countries, infections due to *Vibrio cholerae*, enterotoxigenic *Escherichia coli* (ETEC), rotavirus and *Shigella* are common [2, 8].

In Bangladesh, cholera epidemics are often reported while cases frequently present to the facilities in a moribund state [10]. Shigellosis has a worldwide distribution but predominates mostly in developing countries [14]. A previous study in Bangladesh reported bacterial pathogens such as *V. cholerae*, ETEC, and *Shigella* as the most important causes of diarrhoeal illnesses in adults [2]. On the other hand, rotavirus is the most important aetiological agent of diarrhoea in infants and young children worldwide [11, 15], although this pathogen may also cause dehydrating diarrhoea in adults [15].

There is lack of information on the distribution of most common enteric pathogens, clinical features and disease severity in adult populations. There is also limited information on the distribution of diarrhoeal pathogens in adults, which may be different between urban and rural adult populations [2, 11]. We, therefore, conducted this study to describe the four common aetiologies, socio-demographic status, clinical characteristics of adults presenting with diarrhoea and some or severe dehydration to two urban and two rural diarrhoea treatment facilities in Bangladesh.

## METHODS

### Source of data and Hospital Surveillance System

The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) operates a Diarrhoeal Disease Surveillance System (DDSS) at two urban and two rural treatment facilities, to collect demographic information, presenting features, and severity of disease, and associated morbidities from patients systematically enrolled in the system. A 2% (every 50th patient) subsample from Dhaka Hospital and 10% (every 10th patient) from Mirpur Treatment Centre were enrolled systematically; whereas, all patients coming from the Health and Demographic Surveillance System (HDSS)

area in Matlab and Mirzapur were included irrespective of age, sex, and disease severity. Faecal specimens of these patients underwent laboratory testing (microscopy, ELISA, culture) for detection of enteric pathogens.

Dhaka Hospital, established in 1962, provides cost-free care and treatment to the urban population of Dhaka, the capital of Bangladesh. This hospital currently treats around 140 000 patients with diarrhoea annually, with or without complications, and with or without associated health problems. The hospital has maintained a DDSS since 1979, and systematically enrolls every 50th patient (since 1996) reporting to the triage area, regardless of age, sex, and disease severity.

Mirpur Treatment Centre is a 60-bed new urban hospital (established in 2009) and a relatively small facility located in Mirpur, a community in the north-west of Dhaka city. Around 15 000 patients are treated annually at this urban facility, and 10% (every 10th patient) of these patients are systematically enrolled in the surveillance system.

Kumudini Women's Medical College and Hospital (KWMCH) was established in 1938 in rural Mirzapur subdistrict (Tangail district), located about 60 km north-west of Dhaka. It is a charitable institution providing low-cost health services to the surrounding rural population. The hospital has separate outpatient and inpatient diarrhoea treatment units, and 20–25 inpatient beds to treat nearly 1500 diarrhoeal patients each year. To facilitate the present hospital-based prospective study, an active DDSS was established at the KWMCH. Moreover, the study enrolled all patients among the residents of the regional Demographic Surveillance System for a better understanding of the population-based disease burden.

Matlab Hospital of icddr,b was established in 1963. Matlab is a rural site located 57 km southeast of Dhaka. This hospital provides free care and treatment to 15 000–20 000 diarrhoeal patients living in the HDSS areas and other adjoining subdistricts, each year. All patients with diarrhoeal illnesses coming from the HDSS area are enrolled into the hospital surveillance system.

### Definitions

We defined diarrhoea as  $\geq 3$  abnormally loose or watery stools during a 24-h period [16], and dysentery as visible or reported blood in  $\geq 1$  stool [17]. We defined some dehydration as at least two signs/symptoms, including one key (\*) sign present (irritable/less active\*, sunken eyes, dry mucosa, thirsty,

and reduced skin turgor\* [18, 19]). Severe dehydration was defined as the presence of criteria for 'some dehydration' plus one of the following key (\*) signs/symptoms (lethargy or unconsciousness\*, unable to drink\*, and uncountable/absent radial pulse\* [18, 19]).

Body mass index (BMI) was defined as weight in kilograms divided by the square of height in metres ( $\text{kg/m}^2$ ) [20].

### Selection and sample size of study participants

Between January 2010 and December 2011; a total of 5054 patients aged  $\geq 20$  years were enrolled in the surveillance systems at the four study hospitals with the following distribution: 3443 (68%) in two urban hospitals (2061 in Dhaka Hospital, 1382 in Mirpur Treatment Centre) and 1611 (32%) patients in rural hospitals (417 in Mirzapur Hospital, 1194 patients in Matlab Hospital). Among all patients, 42% were aged 20–39 years, 19% were aged 40–59 years and 8% were elderly (aged  $\geq 60$  years) in urban hospitals; for rural hospitals the age distribution was 12%, 12% and 7%, respectively. The sampling distributions are described in Figure 1.

### Specimen collection and laboratory procedure

A single fresh whole stool specimen (at least 3 ml/g) was collected. Samples from Dhaka Hospital were submitted as soon as possible to the central laboratory located in Dhaka. For Matlab, faecal specimens were submitted to Matlab laboratory located in Matlab Hospital campus. All specimens from Mirpur and Mirzapur were transported to the central laboratory in Dhaka within 6 h of collection using a Styrofoam container with cold packs, maintaining a cool temperature (4–8 °C). Additionally, for Mirzapur and Mirpur, a faecal swab was collected from the stool specimen and inserted in Cary–Blair transport medium in a plastic screw-top test tube, each specimen was packed and labelled with the patients' identification number, and date and time of collection of the specimen. Rotavirus antigen [21], ETEC (testing was performed only for Dhaka and Mirzapur sites) [22], *V. cholerae* [16], and *Shigella* spp. [16] were detected using standard methods [23–25].

### Ethical statement

The DDSS of icddr,b is a routine ongoing activity of Dhaka Hospital, Matlab Hospital and Mirpur Treatment Centre which has been approved by the

Research Review Committee (RRC) and Ethical Review Committee (ERC) of icddr,b. Since it is a routine ongoing activity as well as part of standard medical care, at the time of enrolment verbal consent was taken instead of written consent from the caregivers/guardians on behalf of the patients. The information is stored in the hospital database and used for conducting research. The ERC is satisfied with the voluntary participation, maintenance of the rights of the participants and confidential handling of personal information by the hospital physicians and has approved this consent procedure. The surveillance activities in Mirzapur have been approved by the RRC and ERC of icddr,b as a 3-year research protocol. Both informed written consent from adults or guardians, and assent from children aged 11–17 years was obtained during the study period of this research protocol.

### Data analysis

Statistical analyses were performed using SPSS v. 15.5 (SPSS Inc., USA) and Epi Info v. 6.0 (USD, USA). For analysing socio-demographic characteristics, diarrhoeal patients were divided into three age groups [26, 27]: (i) young adults (20–39 years); (ii) middle-aged adults (40–59 years); and (iii) elderly ( $\geq 60$  years), and the number of patients in these groups was 2751, 1564, and 739, respectively. For comparing major diarrhoeal aetiology, patients were categorized under two age groups: (i) 20–59 years as adults (for insufficient aetiological data) and (ii)  $\geq 60$  years as elderly. For categorical variables, differences in the proportion were compared by  $\chi^2$  test and strengths of associations were estimated by calculating the odds ratios (ORs) and their 95% confidence intervals (CIs). A backward, step-wise logistic regression analysis was performed to examine the association between dependent (urban facilities = 1, rural facilities = 0) and independent variables with the probability of exclusion at  $P = 0.10$ . For this analysis, we combined the two urban facilities and similarly the two rural facilities, for better understating of the urban–rural differentials in diarrhoeal diseases in adults and the elderly.

## RESULTS

The proportion of young adults (20–39 years) in all patients presenting with diarrhoea was higher in urban than rural areas (62% vs. 38%,  $P < 0.001$ ). However, middle-aged (39% vs. 27%,  $P < 0.001$ ) and elderly (22% vs. 11%,  $P < 0.001$ ) more frequently

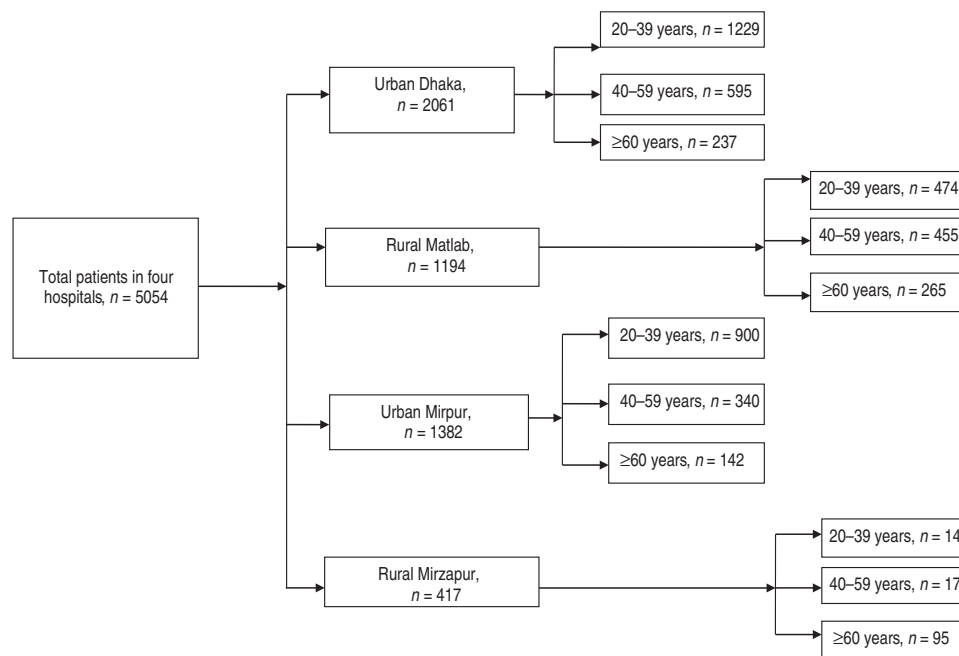


Fig. 1. Sampling frame.

presented to rural facilities than their urban counterparts. A single pathogen was identified in 28% of all specimens tested, where *V. cholerae* was the most commonly detected pathogen (16.4%) followed by rotavirus (4.7%), ETEC (4.4%), and *Shigella* (3.9%).

### Clinical features

For analyses of clinical features, patients were stratified into three age groups: young, middle-aged and elderly. Fever and abdominal pain were more often reported by rural patients than their urban counterparts (21% vs. 2%,  $P < 0.001$ ; 78% vs. 54%,  $P < 0.001$ ), whereas watery diarrhoea was more often reported by urban patients (98% vs. 91%,  $P < 0.001$ ) than patients in rural facilities. Diarrhoea duration was similar in urban and rural sites; however, some or severe dehydration was more frequently observed in young adult urban patients (20–39 years) followed by middle aged (40–59 years) and the elderly. The use of antimicrobials before attending the hospitals was higher in all three age groups of urban patients than the rural patients (Table 1). Other socio-demographic characteristics of study individuals are described in Table 1.

### Comparisons of pathogens between rural and urban facilities

A higher proportion of all patients presenting with diarrhoea at urban facilities had an aetiology of

*V. cholerae* (19% vs. 11%,  $P < 0.001$ ), ETEC (9% vs. 4%,  $P < 0.001$ ), and rotavirus (5% vs. 3%,  $P = 0.013$ ) than at rural facilities. *Shigella* was more commonly detected in rural facilities compared to urban facilities (7% vs. 2%,  $P < 0.001$ ). In urban hospitals, *S. flexneri* (1.4%) was the most common serogroup followed by *S. boydii* (0.6%), and *S. sonnei* (0.3%). In rural facilities, *S. flexneri* (4.9%) was also most common, followed by *S. boydii* (0.9%), *S. sonnei* (0.6%), and *S. dysenteriae* (0.3%) (Table 2).

### Comparisons of pathogens between adult and elderly patients

The isolation rate of *V. cholerae* was higher in all young and middle-aged adult patients (20–59 years) than the elderly (18% vs. 10%,  $P < 0.001$ ). Moreover, the isolation rate of *V. cholerae* was higher in urban adults (20–59 years; 20% vs. 12%,  $P < 0.001$ ) and the elderly (20% vs. 12%,  $P = 0.082$ ) than in the rural population. The isolation rate of *Shigella* was higher in the elderly than in adults irrespective of their age (8% vs. 3%,  $P < 0.001$ ) and it was more often isolated in rural adults (20–59 years), and the elderly than the urban patient population. The isolation rates of *S. flexneri*, *S. boydii*, *S. sonnei* and *S. dysenteriae* were 8.6%, 1.1%, 0.6% and 0.2%, respectively in the rural elderly patient population. In rural adults, the isolation rates of *S. flexneri*, *S. boydii*, *S. sonnei* and *S. dysenteriae* were 4%, 0.9%, 0.7% and 0.2%,

Table 1. Socio-demographic and clinical characteristics of young adults, adults and elderly patients in urban and rural healthcare facilities, Bangladesh, 2010–2011

Characteristic	Young adults, 20–39 years			Middle aged, 40–59 years			Elderly, ≥60 years		
	Urban, n = 2129 (%)	Rural, n = 622 (%)	P value	Urban, n = 935 (%)	Rural, n = 629 (%)	P value	Urban, n = 379 (%)	Rural, n = 360 (%)	P value
Male sex	1170 (54.9)	206 (33.1)	<0.001	496 (53.0)	257 (40.9)	<0.001	210 (56.0)	170 (47.2)	0.021
Illiteracy of patients	569 (26.7)	80 (12.9)	<0.001	459 (49.1)	269 (42.8)	0.016	245 (65.3)	210 (58.3)	0.060
Monthly family income, <US\$100	824 (38.7)	302 (48.6)	<0.001	291 (31.1)	311 (49.4)	<0.001	117 (31.2)	161 (44.7)	<0.001
Body mass index (<18.5 kg/m <sup>2</sup> )	534 (25.1)	127 (20.4)	0.019	162 (17.3)	123 (19.6)	0.292	89 (23.7)	119 (33.1)	0.006
Use of sanitary toilet	1593 (74.8)	197 (31.7)	<0.001	711 (76.0)	214 (34.0)	<0.001	274 (73.1)	120 (33.3)	<0.001
Fever (temperature ≥37.8 °C)	40 (1.9)	119 (19.1)	<0.001	20 (2.1)	139 (22.1)	<0.001	9 (2.4)	77 (21.4)	<0.001
History of abdominal pain	1185 (55.7)	506 (81.4)	<0.001	508 (54.3)	495 (78.7)	<0.001	179 (47.7)	261 (72.5)	<0.001
Watery stool (lack of mucus/blood)	2083 (97.8)	564 (90.7)	<0.001	923 (98.7)	579 (92.1)	<0.001	373 (99.4)	320 (88.9)	<0.001
Duration of diarrhoea (>1 day)	2026 (95.2)	588 (94.5)	0.597	863 (92.3)	574 (91.3)	0.518	350 (93.3)	329 (91.4)	0.393
Number of passed loose stools (>10 times in last 24 h)	1221 (57.4)	287 (46.1)	<0.001	552 (59.0)	302 (48.0)	<0.001	228 (60.8)	181 (50.3)	0.005
Dehydration (some or severe)	1908 (89.6)	394 (63.3)	<0.001	850 (90.9)	390 (62.0)	<0.001	343 (91.5)	234 (65.0)	<0.001
Received antibiotic prior to hospital visit	602 (28.3)	96 (15.4)	<0.001	269 (28.8)	126 (20.0)	<0.001	109 (29.1)	76 (21.1)	0.016

respectively. The isolation rate of ETEC was higher in urban adults than for those in rural areas (Table 2).

**Multivariate analysis**

Significant associations were found between areas and patients’ age, male sex, illiteracy of patients, use of non-sanitary toilet, dysentery, duration of diarrhoea at home before visiting a health facility, dehydration status, fever, abdominal pain and use of antimicrobials at home. The significance of these associations were retained in multivariate analysis after controlling for the potential confounders such as monthly family income (>US\$100), BMI, and number of watery/loose stools (>10 times) (Table 3). Patients presenting with diarrhoea at rural facilities were more likely to be middle aged and elderly. Clinical features like fever, dysentery, and abdominal pain were more often reported by rural patients compared to urban facility patients. Moreover, rural patients used non-sanitary toilets more often than their urban counterparts (Table 3).

**Antibiotic susceptibility**

Of the two most frequently used antimicrobials for treating cholera cases, 85% of the *V. cholerae* isolates in Mirzapur and 84% in Mirpur were susceptible to azithromycin compared to 68% of the isolates in Dhaka. For ciprofloxacin, 88% of the *V. cholerae* isolates in Mirzapur and all isolates (100%) in Dhaka, Matlab, and Mirpur were susceptible. In Mirzapur, nearly 84% of *Shigella* isolates were susceptible to ciprofloxacin and mecillinam – both of these antimicrobials were often used for treating shigellosis. Between 10% and 61% of the isolates were susceptible to other antibiotics such as ampicillin, trimethoprim-sulfamethoxazole (TMP-SMX), and nalidixic acid (Table 4). In Dhaka, 62% and 70% of *Shigella* isolates were susceptible to ciprofloxacin and mecillinam, respectively; while in Matlab susceptibility of *Shigella* isolates to these antibiotics was 68% and 78%, respectively, and in Mirpur the figures were 78% and 67%, respectively (Table 4).

**DISCUSSION**

We observed that the proportion of young adult patients (aged 20–39 years) was higher in urban diarrhoeal disease treatment facilities than in rural facilities. Rapid urbanization and rural to urban

Table 2. Isolation of pathogens in adult diarrhoeal patients in urban and rural healthcare facilities, Bangladesh, 2010–2011

Aetiological agents	Elderly ( $\geq 60$ years)		Adults (20–59 years)		<i>P</i> value <sup>a</sup>	<i>P</i> value <sup>b</sup>	<i>P</i> value <sup>c</sup>	<i>P</i> value <sup>d</sup>
	Urban, <i>n</i> = 379 (%)	Rural, <i>n</i> = 360 (%)	Urban, <i>n</i> = 3064 (%)	Rural, <i>n</i> = 1251(%)				
<i>Shigella</i>	18 (4.7)	39 (10.8)	69 (2.3)	71 (5.7)	0.002	<0.001	0.005	0.001
<i>S. flexneri</i>	11 (2.9)	31 (8.6)	39 (1.2)	48 (3.8)	0.001	<0.001	0.012	<0.001
<i>S. sonnei</i>	3 (0.8)	2 (0.6)	6 (0.2)	9 (0.7)	1.00	0.007	0.056	1.00
<i>S. boydii</i>	4 (1.1)	4 (1.1)	18 (0.6)	11 (0.9)	1.00	0.712	0.275	0.755
<i>S. dysenteriae</i>	0	2 (0.6)	6 (0.2)	3 (0.2)	—	0.283	—	0.312
<i>Vibrio cholerae</i>	45 (11.8)	28 (7.7)	612 (19.9)	146 (11.7)	0.082	<0.001	0.001	0.042
ETEC	21/240 (8.8)	12/190 (6.3)	172/1 837 (9.4)	22/644 (3.4)	0.447	<0.001	0.849	0.117
Rotavirus	17 (4.5)	12 (3.3)	165 (5.4)	42 (3.4)	0.541	0.024	0.751	0.899
Mixed pathogens	26 (6.8)	1 (0.3)	285 (9.3)	20 (1.6)	<0.001	<0.001	0.278	0.062
Non-identified/ tested pathogens	253 (66.8)	251 (69.3)	1 823 (59.5)	887 (70.9)	0.407	<0.001	<0.001	0.609

ETEC, Enterotoxigenic *Escherichia coli*.

<sup>a</sup> Comparison between urban and rural facilities for elderly patients aged  $\geq 60$  years.

<sup>b</sup> Comparison between urban and rural facilities for adult patients aged 20–59 years.

<sup>c</sup> Comparison between all adults and elderly patients attending urban diarrhoeal facilities.

<sup>d</sup> Comparison between all adults and elderly patients attending rural diarrhoeal facilities.

migration for better job opportunities leading to a higher proportion of younger population in this stratum [28], as well as greater exposure of this group to potentially contaminated water and food consumed outside of the home are possible explanations.

In the rural areas, the proportion of females (being more susceptible to disease as well as its severity because of compromised immunity as well as more exposure during active participation in patient care) was higher than males, but the opposite was the case in the urban treatment centres. A similar observation was found in a previous study in urban Bangladesh with poor healthcare behaviour [29], which might be the factor of gender dissimilarity between the rural and urban facilities in the present study.

The isolation frequency of *V. cholerae* was higher in adults (20–59 years) than the elderly. However, shigellosis was more frequent in the elderly compared to adults. Poor personal hygiene practices and an inadequate sanitation system are known risk factors for both *Shigella* and *V. cholerae* [30, 31], but *Shigella* was more prevalent in rural elderly as opposed to *V. cholerae* in urban adults. We do not have a ready explanation for this observation, although it may be that a higher proportion of the elderly population in rural areas with a more altered immune status due to increasing age could contribute to this observation [32]. Additionally, among elderly individuals it is

mostly females that participate in the active care of young grandchildren at the household level, these are often highly susceptible to shigellosis in endemic Bangladesh [33–35]. While contaminated water is the principal vehicle of transmission of *V. cholerae*, contaminated food and person-to-person transmission are the more likely the modes of transmission of *Shigella*. However, our data could not support the analyses to test these possibilities and this issue should be addressed in carefully conducted future studies.

Earlier studies have reported a higher incidence of dysentery or shigellosis in populations aged >40 years [36], and a higher bacterial load in stool and complications due to compromised integrity of intestinal mucosa and host defence [37]. Increased susceptibility to enteric infections due to altered intestinal function in the elderly has also been reported [12]. Infectious diarrhoea is also associated with altered immune functions (such as decreased helper T-cells and their function, decreased mucosal IgA and antibody-forming potential, and increased numbers of gut coliforms) as a consequence of advancing age [2, 38]. Our finding of a higher frequency of shigellosis in the elderly, a population increasing globally including developing countries like Bangladesh, and its higher morbidity will be important in monitoring and identifying risk factors to consider interventions for prevention of this illness in the elderly [39].

Table 3. Characteristics of patients between urban and rural healthcare facilities, Bangladesh, 2010–2011

Characteristic	Adjusted OR (95% CI)	P value
Age (0 = 20–39 years, 1 = 40–59 years, 2 ≥ 60 years)		
20–39 years	—	
40–59 years	0.28 (0.23–0.35)	<0.001
≥60 years	0.15 (0.11–0.20)	<0.001
Male sex (1 = male, 0 = female)	2.32 (1.93–2.78)	<0.001
Illiteracy of patients (1 = illiterate, 0 = literate)	3.02 (2.46–3.71)	<0.001
Use of non-sanitary toilet (1 = non-sanitary toilet, 0 = sanitary toilet)	0.07 (0.06–0.08)	<0.001
Presence of fever (1, ≥37.8 °C; 0, <37.8 °C)	0.05 (0.03–0.07)	<0.001
History of abdominal pain (1 = yes, 0 = no)	0.35 (0.29–0.43)	<0.001
Dysentery (1 = dysentery, 0 = non-dysentery)	0.22 (0.14–0.35)	<0.001
Duration of diarrhoea (1, >1 day; 0, ≤1 day)	1.67 (1.37–2.04)	<0.001
Dehydration (1 = some or severe, 0 = none)	6.57 (5.23–8.27)	<0.001
Use of antimicrobials at home (1 = yes, 0 = no)	1.79 (1.43–2.24)	<0.001

OR, Odds ratio; CI, confidence interval.

Outcome variables: age, male sex, illiteracy of patients, use of non-sanitary toilet, presence of fever, history of abdominal pain, dysentery, duration of diarrhoea, dehydration, and use of antimicrobials at home.

Main exposure: study area, urban vs. rural (diarrhoeal hospital facilities were the reference category).

Adjusted factors: monthly family income, body mass index, and frequency of stool (>10 times in last 24 h).

Although the prevalence of shigellosis has been falling in Bangladesh over the last three decades [40], it is still considered as an endemic zone [1, 41, 42].

In the present study, antibiotic use at home was higher in people attending urban facilities, irrespective of their age group. Overall, 53–75% of the *V. cholerae* isolates were susceptible to tetracycline. In Dhaka and Matlab, all isolates (100%) and in Mirzapur 88% of the strains were susceptible to ciprofloxacin. A previous study reported that multiple resistance to bacterial enteric pathogens and the genetic potency for antibiotic resistance development in the pathogen made the choice of efficient drugs difficult for the treatment of diseases caused by *V. cholerae*, which might also be true for our study [43]. *Shigella* isolates were more susceptible to ciprofloxacin in Mirzapur than the other three facilities; whereas, the susceptibility pattern of another antimicrobial, mecillinam, was identical in the four facilities – these two drugs are most often used for treating shigellosis [44]. Irresponsible use of antimicrobials because of easy availability of antimicrobials over the counter, genetic mutation of the pathogens, host–pathogen–environment interactions, increasing minimum inhibitory concentration (MIC) level of antimicrobials against certain pathogens, enhanced translocation of pathogens in the intestine due to malnutrition as well as its impact on the immune system lead to diverse susceptibility to antimicrobials. Moreover, changes in distribution of serotypes of pathogens, high population density leading to close

contact with livestock, and high selective pressure as a result of unrestricted use of antimicrobials in veterinary medicine, poultry and aquaculture in Bangladesh and other developing countries [45–48].

In the present study, ETEC was isolated more often from urban adult patients aged 20–59 years than rural patients, such higher isolation in these individuals could be due to their frequent exposures to contaminated environment and inadequate hygiene practices [49]. A previous study reported that the incidence of ETEC infections in developing countries decreases after age 5 years, is lowest in people aged 5–15 years [49], and rises again to about 25% in adults [49, 50]. These observations corroborate with our study findings. In hospitalized Bangladeshi patients, severity of ETEC diarrhoea was greater in children and infants [49], and ETEC, in terms of severity, was next to *V. cholerae* [2]. The reasons for this may be due to both environmental and behavioural factors such as the result of ingestion of contaminated food or drink which may predispose to ETEC infection [49, 51]; however, it was also true for the other three pathogens [24, 30, 52]. A previous study stated that the infective dose of bacteria that an adult ingests is much higher than that of a young child, thus leading to severity of diseases being related to the size of the ingested inoculum [53], which could also be true for elderly people.

In case of rotavirus diarrhoea, the incidence was similar in elderly patients of both rural and urban

Table 4. Susceptibility pattern of *Shigella* and *Vibrio cholerae* isolated in different healthcare facilities, Bangladesh, 2010–2011 (aged  $\geq 20$  years)

	Mirzapur, n = 34 (%)	Dhaka, n = 441 (%)	P value	Matlab, n = 140 (%)	P value	Mirpur, n = 216 (%)	P value
Susceptibility pattern of <i>Vibrio cholerae</i>							
Tetracycline	18 (52.9)	280 (63.5)	0.297 <sup>a</sup>	105 (75.0)	0.020 <sup>a</sup> /0.016 <sup>b</sup>	140 (64.8)	0.252 <sup>a</sup> /0.806 <sup>b</sup> /0.056 <sup>c</sup>
TMP-SMX	4 (11.8)	11 (2.5)	0.017 <sup>a</sup>	21 (15.0)	0.788 <sup>a</sup> / $<0.001$ <sup>b</sup>	—	—
Erythromycin	12 (35.3)	1 (0.2)	$<0.001$ <sup>a</sup>	1 (0.7)	$<0.001$ <sup>a</sup> /0.424 <sup>b</sup>	—	$<0.001$ <sup>a</sup> /1.00 <sup>b</sup> /-
Ciprofloxacin	30 (88.2)	440 (99.8)	$<0.001$ <sup>a</sup>	140 (100.0)	$<0.001$ <sup>a</sup> /1.00 <sup>b</sup>	216 (100.0)	$<0.001$ <sup>a</sup> /1.00 <sup>b</sup> /-
Azithromycin	29 (85.3)	298 (67.6)	0.050 <sup>a</sup>	1 (0.7)	$<0.001$ <sup>a</sup> / $<0.001$ <sup>b</sup>	181 (83.8)	0.975 <sup>a</sup> / $<0.001$ <sup>b</sup> / $<0.001$ <sup>c</sup>
	Mirzapur, n = 30 (%)	Dhaka, n = 60 (%)	P value	Matlab, n = 80 (%)	P value	Mirpur, n = 27 (%)	P value
Susceptibility pattern of <i>Shigella</i>							
Ampicillin	18 (60.0)	33 (55.0)	0.8216 <sup>a</sup>	33 (41.3)	0.123 <sup>a</sup> /0.149 <sup>b</sup>	15 (55.6)	0.943 <sup>a</sup> /0.853 <sup>b</sup> /0.285 <sup>c</sup>
TMP-SMX	9 (30.0)	18 (30.0)	0.807 <sup>a</sup>	30 (37.5)	0.611 <sup>a</sup> /0.456 <sup>b</sup>	11 (40.7)	0.568 <sup>a</sup> /0.461 <sup>b</sup> /0.943 <sup>c</sup>
Nalidixic acid	3 (10.0)	1 (1.7)	0.106 <sup>a</sup>	19 (23.8)	0.180 <sup>a</sup> / $<0.001$ <sup>b</sup>	—	—
Mecillinam	26 (86.7)	42 (70.0)	0.140 <sup>a</sup>	62 (77.5)	0.422 <sup>a</sup> /0.418 <sup>b</sup>	21 (77.8)	0.492 <sup>a</sup> /0.622 <sup>b</sup> /0.812 <sup>c</sup>
Ciprofloxacin	25 (83.3)	37 (61.7)	0.064 <sup>a</sup>	54 (67.5)	0.076 <sup>a</sup> /0.591 <sup>b</sup>	18 (66.7)	0.249 <sup>a</sup> /0.835 <sup>b</sup> /0.874 <sup>c</sup>
Azithromycin	n.d.	48 (80.0)	—	—	—	24 (88.9)	0.374 <sup>b</sup>
Ceftriaxone	n.d.	56 (93.0)	—	—	—	26 (96.3)	1.00 <sup>b</sup>

TMP-SMX, Trimethoprim–sulfamethoxazole; n.d., not done.

<sup>a</sup> Comparison between Mirzapur and the other three sites.

<sup>b</sup> Comparison between Dhaka, Matlab and Mirpur sites.

<sup>c</sup> Comparison between Matlab and Mirpur sites.



facilities, but higher in patients attending urban facilities. This is probably due to the differences in the distribution of rotavirus types, as group B mostly accounts for adult diarrhoea [11] and may also cause dehydrating diarrhoea in adults [15]. Moreover, a previous study in Dhaka hospital of icddr,b reported on the higher probability of norovirus (43%) in adults aged  $\geq 18$  years [54]. As our study was not designed to look for these pathogens, further studies are needed to isolate pathogens like group B rotavirus and norovirus in the adult population.

The main limitation of our study is that of being a facility-based study as well examining only four enteric pathogens, so our findings may not represent large population data. Moreover, testing for more pathogens, in particular norovirus, may have increased the yield of pathogens identified. However, within this limitation, unbiased sample selection did show some rural–urban differences as well as differences in the distribution of pathogens by age group.

We observed that a higher proportion of patients presenting with diarrhoea were young adult patients (20–39 years) rather than elderly patients. *V. cholerae* was the most commonly isolated pathogen in adults in both urban and rural facilities. The elderly were more vulnerable to *Shigella* infection particularly in the rural population. Cholera and shigellosis are diseases related to poverty, lack of safe water and sanitation. Therefore, our findings indicate that despite economic and other progress made, conditions facilitating transmission of *V. cholerae* and *Shigella* prevail in Bangladesh. Therefore, efforts are needed to improve personal hygiene practices, provision of safe drinking water and better sanitation. Identifying pathogens of diarrhoeal disease may help to better understand appropriate treatment techniques.

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## DECLARATION OF INTEREST

None.

## REFERENCES

1. **Kotloff KL, et al.** Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013.
2. **Faruque AS, et al.** Diarrhoea in elderly people: aetiology, and clinical characteristics. *Scandinavian Journal of Infectious Diseases* 2004; **36**: 204–208.
3. **Bryce J, et al.** WHO estimates of the causes of death in children. *Lancet* 2005; **365**: 1147–1152.
4. **Kosek M, et al.** The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the World Health Organization* 2003; **81**: 197–204.
5. **Parashar UD, et al.** The global burden of diarrhoeal disease in children. *Bulletin of the World Health Organization* 2003; **81**: 236.
6. **Guerrant RL, et al.** Magnitude and impact of diarrheal diseases. *Archives of Medical Research* 2002; **33**: 351–355.
7. **Aggett P, et al.** Zinc and human health. *Nutrition Reviews* 1995; **53**: S16–22.
8. **Farthing MJ** Diarrhoea: a significant worldwide problem. *International Journal of Antimicrobial Agents* 2000; **14**: 65–69.
9. **Liu L, et al.** Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012; **379**: 2151–2161.

10. **Chowdhury F, et al.** Impact of rapid urbanization on the rates of infection by *Vibrio cholerae* O1 and enterotoxigenic *Escherichia coli* in Dhaka, Bangladesh. *PLoS Neglected Tropical Diseases* 2011; **5**: e999.
11. **Fang ZY, et al.** Investigation of an outbreak of adult diarrhea rotavirus in China. *International Journal of Infectious Diseases* 1989; **160**: 948–953.
12. **Kirk MD, et al.** Gastroenteritis and food-borne disease in elderly people living in long-term care. *Clinical Infectious Diseases* 2010; **50**: 397–404.
13. **de Magny GC, et al.** Cholera outbreak in Senegal in 2005: was climate a factor? *PLoS ONE* 2012; **7**: e44577.
14. **Edwards BH** Salmonella and Shigella species. *Clinical Laboratory Medicine* 1999; **19**: 469–487.
15. **Sanekata T, et al.** Human group B rotavirus infections cause severe diarrhea in children and adults in Bangladesh. *Journal of Clinical Microbiology* 2003; **41**: 2187–2190.
16. **WHO.** *Manual for Laboratory Investigations of Acute Enteric Infections*. Programme for control of diarrhoeal disease. Geneva: World Health Organization, 1987.
17. **Ferdous F, et al.** Aetiology and clinical features of dysentery in children aged <5 years in rural Bangladesh <5 years in rural Bangladesh. *Epidemiology and Infection* 2014; **142**: 90–98.
18. **Alam NH, et al.** Treatment of infectious diarrhea in children. *Paediatric Drugs* 2003; **5**: 151–65.
19. **WHO.** *Treatment of Diarrhoea: a Manual for Physicians and Other Senior Health Workers*. Geneva: World Health Organization, 1990.
20. **Oyeyemi AL, et al.** Relationship of physical activity to cardiovascular risk factors in an urban population of Nigerian adults. *Archives of Public Health* 2013; **71**: 6.
21. **Rahman M, et al.** Genetic characterization of a novel, naturally occurring recombinant human G6P[6] rotavirus. *Journal of Clinical Microbiology* 2003; **41**: 2088–2095.
22. **Qadri F, et al.** Enterotoxigenic *Escherichia coli* and *Vibrio cholerae* diarrhea, Bangladesh, 2004. *Emerging Infectious Diseases* 2005; **11**: 1104–1107.
23. **Talukder KA, et al.** Altering trends in the dominance of *Shigella flexneri* serotypes and emergence of serologically atypical *S. flexneri* strains in Dhaka, Bangladesh. *Journal of Clinical Microbiology* 2001; **39**: 3757–3759.
24. **Tanaka G, et al.** Deaths from rotavirus disease in Bangladeshi children: estimates from hospital-based surveillance. *Pediatric Infectious Disease Journal* 2007; **26**: 1014–1018.
25. **Das SK, et al.** Changing emergence of *Shigella* sero-groups in Bangladesh: observation from four different diarrheal disease hospitals. *PLoS ONE* 2013; **8**: e62029.
26. **Ogden CL, et al.** Prevalence of obesity in the United States, 2009–2010. *NCHS Data Brief* 2012; **82**: 1–8.
27. **Das SK, et al.** Lipoprotein status among urban populations in Bangladesh. *Atherosclerosis* 2012; **223**: 454–457.
28. **ME H, et al.** Rural to Urban migration and household living conditions in Bangladesh. *Dhaka University Journal of Science* 2012; **60**: 253–257.
29. **Perveen I, et al.** Prevalence and health-care seeking pattern of patients with functional diarrhoea in an urban community of Bangladesh. *Mymensingh Medical Journal* 2010; **19**: 85–88.
30. **Mandal S, et al.** Cholera: a great global concern. *Asian Pacific Journal of Tropical Medicine* 2011; **4**: 573–580.
31. **Lee LA, et al.** Hyperendemic shigellosis in the United States: a review of surveillance data for 1967–1988. *International Journal of Infectious Diseases* 1991; **164**: 894–900.
32. **Drewnowski A, et al.** Impact of aging on eating behaviors, food choices, nutrition, and health status. *Journal of Nutrition Health and Aging* 2001; **5**: 75–79.
33. **Ahmed F, et al.** Family latrines and paediatric shigellosis in rural Bangladesh: benefit or risk? *International Journal of Epidemiology* 1994; **23**: 856–862.
34. **Boyce JM, et al.** Patterns of *Shigella* infection in families in rural Bangladesh. *American Journal of Tropical Medicine and Hygiene* 1982; **31**: 1015–20.
35. **Twamley K, et al.** UK-born ethnic minority women and their experiences of feeding their newborn infant. *Midwifery* 2011; **27**: 595–602.
36. **von Seidlein L, et al.** A multicentre study of *Shigella* diarrhoea in six Asian countries: disease burden, clinical manifestations, and microbiology. *PLoS Medicine* 2006; **3**: e353.
37. **Sansonetti PJ, et al.** Rupture of the intestinal epithelial barrier and mucosal invasion by *Shigella flexneri*. *Clinical Infectious Diseases* 1999; **28**: 466–475.
38. **Slotwiner-Nie PK, et al.** Infectious diarrhea in the elderly. *Gastroenterology Clinics of North America* 2001; **30**: 625–635.
39. **Sahlen KG, et al.** Health coaching to promote healthier lifestyle among older people at moderate risk for cardiovascular diseases, diabetes and depression: a study protocol for a randomized controlled trial in Sweden. *BMC Public Health* 2013; **13**: 199.
40. **Khatun F, et al.** Changing species distribution and antimicrobial susceptibility pattern of *Shigella* over a 29-year period (1980–2008). *Epidemiology and Infection* 2011; **139**: 446–452.
41. **Khan AI, et al.** Analysis of fecal leukocytes and erythrocytes in *Shigella* infections in urban Bangladesh. *Southeast Asian Journal of Tropical Medicine and Public Health* 2006; **37**: 747–754.
42. **Bennish ML, et al.** Mortality due to shigellosis: community and hospital data. *Reviews of infectious diseases* 1991; **13** (Suppl. 4): S245–251.
43. **Selianskaia NA, et al.** In vitro induction of transmissive resistance to tetracycline, chloramphenicol and ampicillin chloramphenicol in *Vibrio cholera* non-O1/non-O139 serogroups isolated within 1990–2005 [in Russian]. *Antibiotiki i Khimioterapiia* 2012; **56**: 16–21.
44. **Bhattacharya D, et al.** Antimicrobial resistance in *Shigella* – rapid increase & widening of spectrum in Andaman Islands, India. *Indian Journal of Medical Research* 2012; **135**: 365–370.
45. **Shah SQ, et al.** Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming

- environments of Pakistan and Tanzania. *Environmental Science and Technology* 2012; **46**: 8672–8679.
46. **Hasan B, et al.** Antimicrobial drug-resistant *Escherichia coli* in wild birds and free-range poultry, Bangladesh. *Emerging Infectious Diseases* 2012; **18**: 2055–2058.
  47. **Hasan B, et al.** High prevalence of antibiotic resistance in pathogenic *Escherichia coli* from large- and small-scale poultry farms in Bangladesh. *Avian Diseases* 2012; **55**: 689–692.
  48. **Rahman M, et al.** Extended-spectrum beta-lactamase-mediated third-generation cephalosporin resistance in *Shigella* isolates in Bangladesh. *Journal of Antimicrobial Chemotherapy* 2004; **54**: 846–847.
  49. **Qadri F, et al.** Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clinical Microbiology Reviews* 2005; **18**: 465–483.
  50. **Merson MH, et al.** Disease due to enterotoxigenic *Escherichia coli* in Bangladeshi adults: clinical aspects and a controlled trial of tetracycline. *International Journal of Infectious Diseases* 1980; **141**: 702–711.
  51. **Clemens J, et al.** Development of pathogenicity-driven definitions of outcomes for a field trial of a killed oral vaccine against enterotoxigenic *Escherichia coli* in Egypt: application of an evidence-based method. *International Journal of Infectious Diseases* 2004; **189**: 2299–2307.
  52. **Kotloff KL, et al.** Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bulletin of the World Health Organization* 1999; **77**: 651–666.
  53. **Levine MM, et al.** Immunity to enterotoxigenic *Escherichia coli*. *Infect Immun* 1979; **23**: 729–736.
  54. **Rahman M, et al.** Molecular detection of noroviruses in hospitalized patients in Bangladesh. *European Journal of Clinical Microbiology and Infectious Diseases* 2010; **29**: 937–945.