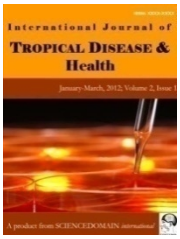

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Disease Severity and Common Etiology of Diarrhea among Children Under-five in Mirzapur, Rural Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Author SKD designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors SA, FF, FDF managed field activities and data collection. Author MJC reviewed and edited the manuscript. Authors KAT, MR, SN, YAB, FQ managed laboratory protocols and activity. Authors GK, TA reviewed and edited the manuscript. Authors ASGF, FQ, KAT and MR contributed to study design, and development of protocol. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aim: There is lack of information on the severity of diarrheal disease with etiology. Thus the study aimed to compare the etiology of under-five children with moderate-to-severe disease (MSD) and mild disease (MD).
Study Design: Diarrheal disease surveillance.
Place and Duration of Study: Mirzapur Kumudini Hospital, Tangail, rural Bangladesh, January 2010 – December 2011.
Methodology: Overall, 2,324 under-5 diarrhea children were enrolled in the hospital who

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came from the demographic surveillance system (DSS) catchment area. Whole stool samples were collected from each enrolled child to detect rotavirus, *Shigella,* ETEC and *V. cholerae.* Information on socio-demographic and clinical characteristics was also collected.

Results: Among all the study children, 1,098 (47%) were aged 0-11 months; 789 (34%) were 12-23 months, and 437 (19%) were 24-59 months. Rotavirus (33%) was mainly responsible for diarrhea amongst children under-5 and 90% of them were less than 2 years. *Shigella* represented 14%; of which, 45% were 24-59 months old. However, ETEC and *V. cholerae* represented only 3% and 2% respectively. *Shigella* was the most commonly detected pathogen (27%) for MSD followed by rotavirus (16%). Conversely, rotavirus (43%) was responsible for MD. MSD were most likely to be infected with *Shigella flexneri* [OR-9.81; 95% CI (6.38, 15.18)] and *Shigella sonnei* [6.29; (3.67, 10.87)] compared to their counterparts with MD. In logistic regression analysis, *Shigella* was responsible for a 2.25 times higher risk for MSD. Children with *Shigella* were 3.28 times at higher risk for bloody stool and 2.45 times more likely to have fever. However, rotavirus diarrhea was more likely to be presented with vomiting (OR-2.46) and fever (OR-1.28), and *Vibrio cholerae*, most often with watery diarrhea (OR-4.35). None of the clinical features were significantly associated with ETEC.

Conclusion: *Shigella* was the leading pathogen that was detected most often in MSD, whereas rotavirus was often associated with MD.

Keywords: Bangladesh; diarrhea; moderate-to-severe disease; rural; under-5 children.

1. INTRODUCTION

Although, the global burden of mortality amongst children under the age of 5 due to diarrhea has decreased, approximately 0·751 million children are still thought to die as a result. Therefore, it is considered the second leading cause of global childhood mortality (uncertainty range, 0·538-1·031) [1]. Poor sanitation, inadequate hygiene, unsafe drinking water, and overcrowding are dominant risk factors. All these enhance the populations' susceptibility [2,3] to be infected with common etiologic agents such as; rotavirus, *Shigella* spp., ETEC and *Vibrio cholerae* [4-7]. Impairment of physical and mental development adds to the burden of Disability Adjusted Life Years (DALYs) lost in addition to nutritional faltering, and breach in the productivity at adulthood [8,9]. Although under-5 child mortality has decreased approximately by half in the past 20 years, there is no indication that disease morbidity is decreasing [2].

Three quarters of global childhood diarrheal deaths occur within only 15 countries and Bangladesh is in 7th position with 50,800 annual childhood deaths [10]. Systematic surveillance in two rural health facilities between August 2005 and July 2007 among under-5 children revealed that 12-14% cases of hospitalization in rural Bangladesh were due to rotavirus infection [11]. Moreover, prevalence of shigellosis fell from 8-12% from 1980 to 3% in 2008, amongst diarrheal disease patients in a large facility in Dhaka, the capital city of Bangladesh [12].

Most clinic-based studies were designed without considering the severity of the disease and did not categorize the degree of severity of disease [4-6,9,11-13]. Thus, the present study aimed to identify the common four microbiologic etiologies (*V. cholerae*, *Shigella*, ETEC, and rotavirus) among rural under-5 children; and to compare the clinical and epidemiological findings between moderate-to-severe (MSD) and milder form of the disease. This study also

emphasized patterns of distribution of rotavirus and other common bacterial causes of diarrhea to provide definitive and comprehensive information on the etiology of MSD as well as mild disease (MD) in infants and young children in rural Bangladesh. The study intends to share this information with clinicians, public health personnel, policy makers, vaccine developers and other interested parties with a view to initiate vaccine and/or public health interventions to reduce burden of disease thereby childhood mortality in rural Bangladesh.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted in the Kumudini Hospital, one of the oldest and largest tertiary hospitals in rural Mirzapur under Tangail district. It is located 60 kilometers north-west of Dhaka, the capital city. The facility has 750 beds and separate in-patient and out-patient units for cases presenting with diarrheal illnesses. The diarrheal disease surveillance system operates round the clock to obtain information and fecal specimens from the residents of Demographic Surveillance System (DSS) area who are reporting with diarrheal illnesses regardless of disease severity.

2.2 Study Population and Inclusion Criteria

Children less than 5 years, residing in the DSS area were the study population. Under-5 children irrespective of sex and socio-demographic status presented in the Kumudini Hospital with history of diarrhea were enrolled in the study without considering severity and duration. Unwilling to participate or refusal to give consent or failure to provide whole stool were excluded.

2.3 Specimen collection and laboratory procedure

A single, fresh, whole stool specimen (at least 3 ml or grams, ideally 10 ml or grams) was collected from all children aged 0-59 months enrolled in Kumudini Hospital, Mirzapur. A faecal swab was then placed in Cary-Blair medium in a plastic screw top test tube. Stool samples were then transported to the central laboratory in Dhaka Hospital for screening common enteric pathogens such as ETEC [14], *Vibrio cholerae* [15], *Shigella* spp. [15], and rotavirus [16] maintaining standard protocols. Optimal cool temperature was strictly maintained from the point of collection of stool sample to successful submission.

For ETEC, stool samples were plated onto MacConkey agar, and the plates were incubated at 37ºC for 18 hours. Six lactose-fermenting individual colonies, morphologically resembling *E. coli*, were tested [13].

For *Vibrio cholerae*, stool sample was plated on taurocholate-tellurite-gelatin agar [17] (Difco, Detroit, Mich.); after overnight incubation of plates, serological confirmation of suspected vibrio colonies was carried out by slide agglutination [18,19] .

Shigella species were isolated and identified in the enteric microbiology laboratory by using standard biochemical and microbiological methods (WHO, 1987) [15]. Stool specimens were inoculated in MacConkey and *Shigella-Salmonella* agar plates and incubated overnight at 37ºC. Nonlactose-fermenting colonies; characteristically resembling *Shigella,* were inoculated into Kligler's iron agar tube for typical reaction, mannitol fermentation, citrate

utilization, urease and indole production, and lysine decarboxylation. *Shigella* sero-types were confirmed by slide agglutination with polyvalent somatic (O) antigen grouping sera, followed by testing with monovalent antisera for specific serotype identification (Denka Seiken, Japan). In cases where no agglutination occurred with live bacteria, 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 [20].

Group A rotavirus-specific VP6 antigen was detected in the stool specimens using solidphase sandwich-type enzyme immunoassay modeled according to the commercial kit [UTF-8 ProSpecT Rotavirus Microplate Assay (Oxoid Ltd Wade Road Basingstoke Hants, UK)]. Positive and negative controls were included in every test run. Quality control of the Enzyme Immune Assay test was routinely done using rotavirus positive samples with known Optical Density values [16] .

2.4 Definition

Diarrhea was defined as passage of three or more abnormally loose or watery stool in a 24-hour period. Dysentery was defined as at least one loose stool containing blood in a 24-hour period. Moderate-to-severe disease (MSD) was defined as if any of the following were present as is the standard definition of MSD in Global Enteric Multicenter Study; and the criteria were presence of - sunken eyes and/or wrinkled skin and/or visible or reported blood in stool; or a child was hospitalized with diarrhea or dysentery; or a child needed intravenous rehydration [21-23]. Children below 5 years without any signs of MSD (sunken eyes and/or wrinkled skin and/or visible or reported blood in stool; or a child was hospitalized with diarrhea or dysentery; or a child needed intravenous rehydration) were considered as mild disease (MD).

2.5 Data Management and Analysis

For categorical variables, differences in the proportions were compared by Chi-square test and a probability of <0.05 was considered as statistically significant. Strength of association was determined by estimating odds ratio (crude OR) and its 95% confidence interval (CI). Statistical Package for Social Sciences (SPSS) Windows (Version 15.2; Chicago, IL) and Epi Info (Version 6.0, USD, Stone Mountain, GA) were used for data entry and subsequent analysis. Finally, logistic regression was performed taking into consideration the etiology of diarrhea with disease severity and clinical findings.

3. RESULTS AND DISCUSSION

Overall, infants (0-11 months) comprised 47% of all patients followed by children 12-23 months old (34%), and children 24-59 months (19%). Thirty one percent children were enrolled as MSD. Of them, 22% had sunken eye, 5% had loss of skin tourgor, 17% needed intravenous saline, 76% had dysentery and 36% were hospitalized with diarrhea or dysentery.

Information on socio-economic-demographic characteristics such as sex (61% vs. 61%, p=0.91), maternal literacy (88% vs. 90% 0.073), presence of cemented household floor (23% vs. 26%, p=0.12), immunization with measles vaccine (88% vs. 87%, p=0.66), use of soap after defecation (78% vs. 81%, p=0.13), and practice of water treatment (5% vs. 7%,

p=0.06) were indistinguishable among the MSD and MD except availability of electricity (69% vs. 75%, p=0.01). Moreover, all the indicators were also found similar irrespective of pathogens isolated from MSD and MD cases.

On the other hand, clinical manifestations varied between MSD and MD. MD cases often presented with watery stool. Conversely, MSD cases had high mean temperature, cough and convulsion (Table 1). Mean duration of diarrheal episodes prior to hospital attendance was same for MSD and MD cases (2.63 vs. 2.37 days; p=0.28).

Table 1. Clinical characteristics of patients with MSD and MD in rural Mirzapur (2010-11)

Clinical characteristics	MSD; n=901 (39%)	MD; n=1415 (61%)	Crude OR (95% CI) p value
Simple watery	202 (22)	1376 (97)	0.01 (0.01, 0.01) <.001
Sticky/mucoid	20 (2)	44 (3)	0.71 (0.40, 1.24) .25
Vomiting	355 (39)	906 (64)	0.37 (0.31, 0.44) <.001
Abdominal pain	742 (82)	873 (62)	2.88 (2.34, 3.54) <.001
Straining	689 (76)	407 (29)	7.98 (6.56, 9.71) <.001
Measles (last 1 month)	32 (4)	97 (7)	0.50 (0.33, 0.77) .01
Eye (sunken)	195 (22)	13 (1)	29.77 (16.44, 55.00) <.001
Fever	510 (56)	600 (42)	1.77 (1.49, 2.10) <.001
Cough	385 (43)	667 (47)	0.84 (0.71, 0.99) .042
Convulsion	30 (3)	8 (1)	6.06 (2.65, 14.39) <0.001
Mean temperature (mean±SD)	37.4±0.9	37.1±0.7	(-0.31, -0.17) <0.001
Respiratory rate (mean±SD)	33.9±5.6	33.8±4.3	(-0.57, 0.29) .53

Rotavirus (33%) was the commonly identified diarrheal pathogen among children less than five years with 90% of them were aged less than two years. On the other hand, *Shigella* represented 14% of total children and children aged 24-59 months (45%) predominantly suffered from shigellosis. However, ETEC and *V. cholerae* represented 3% and 2% young childhood diarrheal illnesses respectively. Proportion of children having mixed pathogen was found only in 3% children (Table 2).

Table 2. Age specific infection rate among under-5 children in rural, Mirzapur (2010-11)

Age group, n=2324 (%)	ETEC n=78 (3%)	Rotavirus n=756 (33%)	*Shigella* n=328 (14%)	*V. cholerae* n=46 (2%)	Mixed pathogen n=76 (3%)	No pathogen n=1195 (52%)
0-11 months, 1098 (47)	28 (37)	387 (51)	57 (17)	15 (33)	33 (43)	644 (54)
12-23 months, 789 (34)	37 (49)	295 (39)	122 (37)	19 (41)	32 (42)	351 (29)
24-59 months, 437 (19)	11 (14)	74 (10)	149 (45)	12 (26)	11 (15)	200 (17)

In Mirzapur, *Shigella* was the most common enteric pathogen (27%) followed by rotavirus diarrhea (16%) among under-5 children who presented with MSD. In contrast to that, rotavirus was the prime (43%) cause of diarrhea among under-5 children who reported to the

health facility with MD. Conversely, the proportions of children with mixed pathogen as well as no pathogen were identical (Table 3).

Table 3. Distribution of pathogens among under-5 children with moderate-to-severe disease and mild disease in rural Mirzapur (2010-11)

Pathogen	Moderate-to-severe disease; n=904 (%)	Mild disease; n=1420 (%)	Crude OR (95% CI) p value
Rotavirus	148 (16)	608 (43)	0.26 (0.21, 0.32) <.001
Shigella	244 (27)	82 (6)	5.44 (4.14, 7.16) <.001
ETEC	29 (3)	47 (3)	0.97 (0.59, 1.59) .98
Vibrio cholerae	24 (3)	22 (2)	1.73 (0.93, 3.23) .09
Mixed pathogen	32 (4)	44 (3)	1.15 (0.71, 1.87) .63
No pathogen	487 (54)	708 (50)	1.18 (1.00, 1.40) .05

Under-5 children infected with *Shigella flexneri* and *Shigella sonnei* were more common in those under-5 children who presented with MSD compared to their counterparts with mild disease (Fig. 1). However, isolation rates of ETEC and *V. cholerae* were found to be similar in both MSD and MD cases.

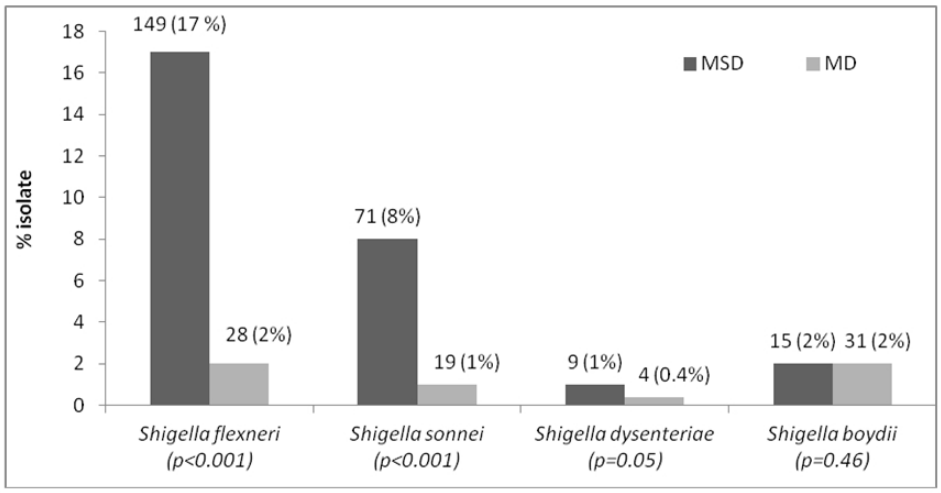

Fig. 1. Distribution of *Shigella* spp. among under-5 children presenting with MSD (n=901) and MD (n=1415) in rural Mirzapur (2010-11)

Shigella was 2.25 times more likely to be detected in MSD than MD among under-5 children. Whereas, presence of ETEC was 7% excess in MSD; however, both rotavirus and *Vibrio choleare* were found to be less common in MSD. Under-5 children with *Shigella* were 3.28 times more likely to have bloody stool and presented 2.45 times more often with fever compared to non-*Shigella* children. On the other hand, for rotavirus, under-5 children more commonly presented with vomiting (OR-2.46) and fever (OR-1.28). Conversely, children infected with *Vibrio cholerae* were at higher risk (OR-4.35) for watery diarrhea. However, none of the clinical features were significantly associated with ETEC diarrhea (Table 4).

Table 4. Association of disease severity and clinical features with etiology of diarrhea among under-5 children in Mirzapur

Indicators	*Shigella;* Adjusted OR (95% CI) p-value	Rotavirus; Adjusted OR (95% CI) p-value	*Vibrio cholerae*; Adjusted OR (95% CI) p-value	ETEC; Adjusted OR (95% CI) p-value
Disease severity (1=MSD, 0=MD)	2.25 (1.20, 4.20) .01	0.88 (0.53, 1.47) .62	0.61 (0.16, 2.43) .48	1.07 (0.33, 3.40) .91
Visible blood in stool (1= Yes, 0=No)	3.28 (1.78, 6.02) <.001	0.09 (0.05, 0.17) <.001	2.95 (0.75, 11.60).12	0.58 (0.17, 1.92) .58
Abdominal pain (1= Yes, 0=No)	0.94 (0.66, 1.34) .74	1.12 (0.89, 1.40) .33	0.75 (0.37, 1.51) .42	0.87 (0.51, 1.49) .61
Some-severe dehydration (1= Yes, 0=No)	0.85 (0.49, 1.49) .58	1.02 (0.43, 2.46) .96	0.96 (0.22, 4.24) .96	1.19 (0.31, 4.52) .79
Sunken eye (1= Yes, 0=No)	0.81 (0.39, 1.71) .58	1.85 (0.77, 4.47) .16	4.35 (0.95, 20.01) .05	1.63 (0.36, 7.42) .53
Fever (1= Yes, 0=No)	2.45 (1.87, 3.23) <.001	1.28 (1.04, 1.58) .02	1.43 (0.75, 2.70) .27	0.89 (0.54, 1.46) .65
Cough (1= Yes, 0=No)	0.69 (0.53, 0.90) .01	1.19 (0.97, 1.46) .09	0.42 (0.21, 0.85) .01	0.77 (0.47, 1.25) .29
Straining (1= Yes, 0=No)	1.22 (0.86, 1.72) .26	0.62 (0.49, 0.78) <.001	0.57 (0.25, 1.26) .16	1.15 (0.66, 2.01) .62
Vomiting (1= Yes, 0=No)	1.18 (0.89, 1.58) .25	2.46 (1.97, 3.06) <.001	0.93 (0.48, 1.80) .83	0.85 (0.51, 1.41) .53

**Dependent variables: Shigella, rotavirus, Vibrio cholerae, ETEC*

4. DISCUSSION

Rotavirus, *Shigella* spp., *Vibrio cholerae*, and ETEC are the known major etiologic agents that are responsible for deaths particularly in children [4-7,24]. The present study facilitates the understanding of the etiology of diarrhea among children below 5 years visiting a large rural health facility. The study revealed that most of the children presented with diarrhea were aged 0-23 months and rotavirus was the leading pathogen. It has been already reported that up to 5 years of age, every child is expected to suffer from an episode of rotavirus diarrhea [24]. Globally, rotavirus causes approximately 111 million episodes of diarrhea and over 400,000 deaths in children under 5 years of age [24]. Although mortality due to rotavirus diarrhea decreased worldwide; rotaviral diarrhea among infant and young children still remains a major public health burden due to higher hospital attendance rate [24,25]. In the present study, it has been observed that children with MD more often visited the hospital with rotavirus infection than the children with MSD. This has been supported by previous reports that mild disease cases substantially became alarming enough, for care seeking in high number at the hospital. Increased use of oral rehydration fluid and zinc with improved general awareness about prompt management of diarrheal illnesses might have resulted in reduced severity of disease [26,27]. At the same time, whilst a reduction of overall prevalence of malnutrition among the under 5 years old children would have impacted on the increase of rotavirus diarrhea; a direct association of rotavirus with malnutrition was seen [28]. Vomiting and fever, the common clinical manifestations of rotavirus were also reported in the present study [29-31], and at least 14% children presented with some-to-severe dehydration and of them, 15% children required rehydration with intravenous fluid followed by ORS [31]. Destruction of gut enterocyte and disruption of reabsorption of water and electrolytes with reduced activity of brush-border membrane bound disaccharidases likely to activate the calcium ion-dependent secretory reflexes of the enteric nervous system resulting in loss of water and electrolytes and children may present with history of frequent passage of watery stool with dehydrating diarrhea [32].

Alternatively, *Shigella* accounted for 14% of total isolates and older children aged 24-59 months old (45%) suffered predominantly. Children with MSD were at 2 times higher risk for shigellosis. It was unexpected that one-third of the children were enrolled as MSD and of them three-fourth had dysentery with presence of blood; either reported or observed by the caregiver. This was surprising and interesting finding; however, we do not have any ready explanation for that, as the socio-demographic characteristics of MSD and MD were similar except availability of electricity. The proportion MSD (35%) and MD (65%) among under 5 chlidren was almost similar as reported by Lamberit LM *et.al.* [33] despite definition of MSD differ from the present study. The present study followed the same definition of MSD of Global Enteric Multicenter Study (GEMS) [21-23] where dysentery was part of the inclusion criteria of MSD due to invasive properties of the pathogens responsible for dysentery specially *Shigella*. It not only causes cellular disruption of the intestinal mucosa [34,35] but also anorexia and malabsorption with compromised immune response [34]. All these features result in acute and chronic adverse consequences including malnutrition.

A study in Pakistan reported that rate of shigellosis increased with advancing age and reached the highest point during the second year of life [36]. In the current study, *Shigella* was responsible for 27% of the MSD cases among children under 5 years of age and 6% in case of young children with MD in rural Mirzapur. Globally, shigellosis accounts for 69% of all episodes and 61% of all deaths attributable to shigellosis affecting children less than 5 years of age [37]. Although the proportion of shigellosis has significantly decreased over the last three decades [12], Bangladesh is still considered as an endemic zone [38]. Shigellosis

is the disease of poor and insolvent people that lack access to proper water and sanitation and do not get appropriate treatment [36]. The burden of the disease is higher in resource poor settings of developing countries and may cause as high as 167 million episodes of diarrhea and more than a million deaths each year [39]. In the present study, *Shigella flexneri and Shigella sonnei* were the two major sero-groups that dominantly caused MSD among rural under-5 children. Children with shigellosis often presented with bloody stool with fever, straining, abdominal pain, and vomiting. Findings from recent past also supported our current observations [40,41].

On the other hand, detection rate of *V. cholerae* was very low in rural Mirzapur among under-5 children and clinically characterized by a more sudden onset; watery diarrhea; and associated dehydration, vomiting, muscle cramps, and abdominal pain which often lead to more severe dehydration requiring immediate hospitalization [42].

4.1 Limitations

The present study focused on the most common pathogens that cause diarrhea among under-5 year old children. There may be other pathogens that may also cause MSD as well as MD. However, the study was able to distinguish the 4 major pathogens responsible for disease severity with an unbiased large sample size in rural resource constraint setting.

5. CONCLUSION

It has become difficult to understand the predictors that influence this diversity of enteropathogens and disease severity due to identical socio-economic-demographic characteristics that are likely to have confounding effects. Other factors like variation of host characteristics of the study children leading to severity of the disease by specific pathogens as well as virulence factors of enteric pathogens to produce the severity were inconclusive. However, the study was able to determine the disease severity specified etiology of diarrhea among children less than 5 years old that may guide the policy makers to understand the necessity to implement interventions including vaccines. The high percentage of *Shigella* infection in this population should make it ideal to test new *Shigella* vaccines, as well as make it useful for clinicians treating these patients.

CONSENT

Written, informed consent was obtained from the parent or primary caretaker of the child with diarrhea who meets all eligibility criteria before any research activities were performed. First, the study explained in local language or dialect. The parent or primary caretaker was given a copy of the consent form to read or share with confidents who were able to read. When illiterate parents were encountered, the consent form translated into the relevant local language was read out for the parents loudly. Thereafter, consent was documented by asking the parent or primary caretaker to sign his/her name (or place an “x” or a fingerprint if unable to sign his/her name) on the consent form. An impartial third party who witnessed the entire consent process signed the consent document. The original signed/imprinted form was retained at the site and the parent or primary caretaker was given a copy to keep. The participating primary caretakers were briefed about the benefit of the study.

ETHICAL APPROVAL

This study was approved by the Research Review Committee and Ethical Review Committee of icddr,b (Protocol number: PR-09064, Grant no-GR-00599). The ERC and RRC of icddr'b have international accreditation in Bangladesh.

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COMPETING INTERESTS

No competing interests exist. All authors confirm that there is no professional affiliation, financial agreement or other involvement with any company whose product figures prominently in the submitted manuscript.

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