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Molecular epidemiology of toxigenic *Clostridioides difficile* isolates from hospitalized patients and the hospital environment in Dhaka, Bangladesh

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Abstract

Epidemiology of *Clostridioides difficile* (syn. *Clostridium difficile*) infection (CDI) in Bangladesh is poorly understood. This study assessed the epidemiology of CDI in hospitalized patients and hospital environmental contamination of toxigenic *C. difficile* at two large urban Bangladesh hospitals. This 12-month prospective observational cohort study collected stool samples from adults with diarrhea and recent antimicrobial exposure during 2017. Environmental samples were collected by swabbing surfaces of hospital common areas. Samples underwent toxigenic culture. *C. difficile* isolates were tested for toxins A and B and PCR-ribotyped. Of 208 stool samples, 18 (8.7%) were positive for toxigenic *C. difficile*. Of 400 environmental samples, 45 (11%) were positive for toxigenic *C. difficile*. Ribotypes present in 10% of stool isolates were 017 (38%), 053–163 (13%), and a novel ribotype (FP435 [13%]). Common ribotypes in environmental isolates were 017 (22%), 053–163 (11%), 106 (24%). This is the first report describing current epidemiology of CDI in at risk hospitalized adult patients in Bangladesh.

Keywords

Clostridium difficile; Developing countries; Asia; Prospective studies

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

1. Introduction

Clostridioides difficile infection (CDI) is the most common cause of infectious diarrhea in hospitalized patients in developed countries and is associated with increased hospitalization duration, costs, and mortality [1,2]. However, little is known about the epidemiology of CDI in resource-limited parts of the world where testing for CDI is not routinely performed [1]. This is most likely due to low clinical suspicion and/or lack of resources or infrastructure for appropriate diagnostic testing [3,4]. However, available reports from resource-limited settings indicate that the prevalence of CDI in these settings is no less than that of North America and Europe [3,5–10]. The prevalence of CDI among acute care hospitalized patients with diarrhea in South Asian countries including India, Pakistan, Singapore, and Thailand, is approximately 10.5% [11]. Yet, in many resource-limited countries including Bangladesh, *C. difficile* is still not routinely tested in diarrheal stool samples, despite widespread use of broad spectrum antibiotics [9]. Thus, CDI is likely an under-recognized cause of acute diarrhea in at risk patients in Bangladesh. An accurate diagnosis of CDI combined with molecular strain typing is essential to implement infection control measures, re-evaluate the need for antimicrobials, and start effective therapy.

The current epidemiology of toxigenic *C. difficile* in Bangladesh is poorly understood as only two studies have reported *C. difficile* from Bangladesh [12,13]. Since then, a previous study demonstrated the presence of toxigenic *C. difficile* in shoe sole swab samples of urban communities in Dhaka, Bangladesh [14]. This previous work also tested 208 diarrheal patients who were admitted to the Dhaka hospital of the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh (icddr,b) for toxigenic *C. difficile*. These 208 patients were a subset of patients who were enrolled in the Diarrheal Disease Surveillance System (DDSS) at icddr,b, in which data (including enteric pathogen data) are collected from every 50th diarrheal patient admitted to icddr,b. Toxigenic *C. difficile* was not detected in any of the 208 patients, most likely because these patients might not have been at risk for CDI (the only inclusion criteria was diarrhea). Dhaka is a city of 15 million inhabitants burdened by other diarrheal diseases; a recent study showed that acute diarrhea in DDSS patients at icddr,b, were mostly due to *V. cholerae*, Rotavirus, and Enterobacteriaceae [15]. Hence, the objective of this study was to assess the epidemiology of CDI in at-risk hospitalized patients and to assess the hospital environment for contamination with toxigenic *C. difficile* in Dhaka, Bangladesh. A secondary objective was to characterize strain distribution of clinical and hospital environmental toxigenic *C. difficile* in Dhaka, Bangladesh.

2. Materials and methods

2.1. Study design and setting

This was a 12-month (January to December 2017) prospective observational cohort study conducted at Bangabandhu Sheikh Mujib Medical University (BSMMU) and Dhaka Medical College and Hospital (DMCH) in Dhaka, Bangladesh. Both are large tertiary care centers (BSMMU has 1500 beds with 48 departments and DMCH has 2300 beds with 34 departments). Prior to this study, testing for CDI was not performed at either hospital. This study was approved by the Research Review Committee and the Ethical Review Committee

at the International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh (icddr,b) and the institutional review boards at the study hospitals.

2.2. Clinical sample collection

Hospitalized adults (≥ 18 years old) with diarrhea (≥ 3 loose stools in a 24-h period) without an identifiable cause and antimicrobial exposure within the past 30 days were eligible for clinical stool sample collection. Patients from the following units of the hospitals were eligible: intensive care units, high dependency units, gastroenterology, palliative care, oncology, hematology, burn intensive care units, and burn high dependency units. Most patients shared a large open ward with many other patients as the number of private rooms are limited. Patient screening was performed by study physicians who went to various hospital units and assessed patients' eligibility by reviewing the medical records and/or interviewing the patients directly. All patients or their legal representatives provided written informed consent before enrollment. Diarrheal stool samples were collected from patients who met the inclusion criteria and gave consent.

2.3. Hospital environmental sample collection

Hospital environmental samples from the same time period were collected by swabbing surfaces of areas commonly used by the public. Surfaces within an arm's reach from patient beds were categorized as "proximal to patient beds", including floor underneath the patient beds, nurses' shoe soles (we assumed nurses would most often be at the bedside), bed railings, intravenous pole stands, and bedside table cabinet handles. Other samples were categorized as either "non-proximal to patient beds" or "restrooms". Restroom samples were categorized separately as we suspected they would be most contaminated with toxigenic *C. difficile*. Samples were obtained from both patient care areas (e.g., general wards, intensive care units, nursing stations) and non-patient care areas (e.g., hospital kitchen, cafeteria, stairway hand-rails). Samples were collected as previously described by swabbing surfaces using pre-sterilized cotton gauze lightly soaked with 0.85% NaCl [16]. Each sample was collected in a 50 ml size pre-sterilized tube. Appropriately trained research staff collected all specimens using sterile gloves. For every 25 swabs, a negative control of 1 swab that was not used but placed with the rest of the swabs was utilized to ensure cross-contamination did not occur.

2.4. Microbiologic procedures

Diarrheal stool samples were initially tested at the microbiology laboratory at BSMMU and the Enteric and Food Microbiology Laboratory at icddr,b using the *C. diff Quik Check Complete* (TECHLAB, Blacksburg, VA) according to the manufacturer's instructions [17]. In a single reaction well, the assay screens for the presence of *C. difficile* by detecting the glutamate dehydrogenase (GDH) antigen and confirms the presence of toxigenic *C. difficile* by detecting toxins A and B in fecal samples. The GDH portion of the assay is excellent at ruling out CDI (98.7% sensitivity). The toxins portion of the assay has 99.4% specificity but is not as sensitive in detecting CDI (87.8% sensitivity). Nevertheless, given the local infrastructure and the availability of the *C. diff Quik Check Complete* in Bangladesh, this diagnostic assay was the most practical in gaining initial insight into the local epidemiology

of CDI [18]. A diagnosis of CDI per the *C. diff Quik Check Complete* required both GDH positivity and toxin positivity [17].

Leftover stool samples were stored at -80°C for further microbiologic testing.

All stool and environmental samples were then sent to a centralized research microbiology laboratory in the United States to undergo toxigenic culture (anaerobic culture plus toxin test) as previously described [16]. Presence of toxin genes was assessed using multiplex polymerase chain reaction (PCR) to detect the presence of toxin A (*tcdA*), toxin B (*tcdB*) [19], and the binary toxin (*cdtA* and *cdtB*) genes [20]. Fluorescent ribotyping was performed as previously described [16,21]. The library contains over 100 known ribotypes (<https://thewalklab.com/tools/>) but does not distinguish between ribotypes 053 and 163, ribotypes 014 and 020, and ribotypes 078 and 126; therefore, these are reported as combined ribotypes (i.e., 053–163, 014–020, and 078–126).

A patient was deemed to have CDI if the stool sample was positive for toxigenic *C. difficile* per the *C. diff Quik Check Complete* (GDH positive and toxin A/B positive) and/or per toxigenic culture. All toxigenic *C. difficile* isolates from environmental samples were identified via toxigenic culture.

2.5. Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows v. 25 (IBM Corp., Armonk, NY). Variables associated with toxigenic *C. difficile* in clinical stool samples were assessed using Mann-Whitney *U* test for continuous variables and Fisher exact test or χ^2 test for categorical variables. Hospital environmental contamination with toxigenic *C. difficile* was compared between sampling sites using χ^2 test. All tests were 2-sided with a significance level of <0.05 .

3. Results

3.1. Epidemiology of CDI in hospitalized patients

A total of 208 stool samples from 196 unique patients were collected during the study period. Of 208 stool samples, 18 (8.7%) samples from unique patients were positive for toxigenic *C. difficile*, the proportion of which was similar between BSMMU (14/165 [8.5%]) and DMCH (4/43 [9.3%]). Each toxigenic isolate was from a unique patient. The majority of stool samples (79%) were collected from BSMMU, and approximately 60% were from male patients (Table 1). On the day of stool sample collection, most patients had been in the hospital for approximately two weeks, had diarrhea for 2–3 days, and were on antibiotics for >1 week. Patients with CDI appeared to have shared an open ward with a greater number of patients compared to patients without CDI. Only 22% of patients with CDI were receiving metronidazole at the time of stool sample collection.

Of the 18 positive cases, 7 were identified based on both toxigenic culture and the *C. diff Quik Check Complete*; 9 were identified based on toxigenic culture only (these 9 isolates were GDH negative and toxin A/B negative per the *C. diff Quik Check Complete*), and 2 were identified based on the *C. diff Quik Check Complete* only (these 2 isolates could not be

recovered in the centralized laboratory). The 16 isolates that were recovered using toxigenic culture in the centralized laboratory underwent ribotyping.

3.2. Epidemiology of toxigenic *C. difficile* in the hospital environment

A total of 400 environmental samples were collected (BSMMU = 253, DMCH = 147) during the same study period. Overall, 45 (11%) environmental samples grew toxigenic *C. difficile*, with similar rates of positivity observed between the two hospitals (13% at BSMMU vs 9% at DMCH). Toxigenic isolates were somewhat more prevalent in patient care areas than non-patient care areas (13% vs 7%, $p = 0.053$). Restrooms and surfaces proximal to patient beds were more likely to be contaminated with toxigenic *C. difficile* than surfaces not proximal to patient beds, $p < 0.001$ (Fig. 1). Besides restrooms, specific sites that were most contaminated (>10% of samples from that site were positive for toxigenic *C. difficile*) included nursing stations (25%), bed railings (24%), floor underneath patient beds (18%), nurses' shoe soles (18%), and bedside table cabinet handles (11%).

3.3. Ribotypes of clinical stool isolates vs hospital environmental isolates

Ribotyping was performed on 16 toxigenic clinical isolates and 45 toxigenic environmental isolates. These isolates were positive for toxin A and B by PCR and negative for binary toxin. Ribotypes present in 10% of stool isolates were 017 (38%), 053–163 (13%), and a novel ribotype not present in the library (designated FP435; 13%) (Fig. 2). Four stool isolates had ribotypes novel to our database library. A similar distribution was seen in hospital environmental isolates with one exception. Ribotypes 017 and 053–163 were observed in 10% environmental isolates. However, ribotype 106 was more common in environmental isolates than in stool isolates (24% vs 6%). Ribotypes 017 and 106 were the most common environmental ribotypes at both hospitals. Seven of 45 environmental isolates had ribotypes novel to our database library. Ribotypes 106 and 053–163 were more common in areas non-proximal to patient beds, while 017 was more common in areas proximal to patient beds.

4. Discussion

This study demonstrated that the prevalence of CDI in at risk adult hospitalized patients (patients with diarrhea with no other identifiable cause and recent antimicrobial exposure) in two large hospitals in Bangladesh was 9%. This prevalence is similar to that reported in other South Asian countries and the United States [11,22,23]. Clinicians at these two hospitals were not testing for *C. difficile* until this study was conducted, highlighting the importance of this under-diagnosis. This finding contrasts the findings of a previous study which found no toxigenic *C. difficile* among 208 hospitalized diarrheal patients in Dhaka, Bangladesh [14]. As explained previously, the most likely explanation is that this study targeted patients who were at higher risk for CDI.

In this study, toxigenic culture did not identify two cases of CDI that were initially identified by the *C. diff* Quik Check Complete, perhaps due to difficulties during processing and shipping. However, this study does demonstrate that worldwide molecular surveillance of *C. difficile* is possible with good recovery rates of *C. difficile*, most likely due to presence of

hardy spores in the shipped samples. Of the other 16 cases identified by toxigenic culture, 9 were not identified by the *C. diff Quik Check Complete*. This discrepancy is likely due to the high sensitivity of toxigenic culture (anaerobic culture plus toxin A/B/binary gene PCR) compared to GDH and toxin A/B enzyme immunoassays [2,24]. However, the ease of use made the *C. diff Quik Check Complete* practical to use on site for this study. Further studies will be required to identify the most practical diagnostic tool to identify CDI in Bangladesh hospitals.

On average a patient shared an open ward with 14 other patients (Table 1), and we observed a trend that patients with CDI (vs patients without CDI) were more likely to share a ward with a greater number of patients. This is consistent with the findings of previous studies which showed a positive association between the number of hospital roommate exposures and acquisition of CDI [25,26]. More patients means more “traffic” from healthcare providers and visitors, which can increase the risk of indirect transmission. Since amoebiasis and other intestinal parasitic infections are endemic in Bangladesh, metronidazole is often given empirically to patients with diarrhea and may unintentionally treat CDI [27]. However, after having diarrhea for 2–3 days, only 22% of patients with CDI in this study were receiving metronidazole at the time of stool collection, which was similar to the proportion of non-CDI patients with diarrhea who received metronidazole. This suggests the majority of patients with undiagnosed CDI could have been left untreated for a period of time. The clinical significance of this delay or under treatment will require further study.

Eleven percent of hospital environmental samples were positive for toxigenic *C. difficile*. This is similar to the results of two previous studies which detected toxigenic *C. difficile* in 10% of community environmental samples from Dhaka, Bangladesh and 16.5% of hospital environmental samples from Houston, Texas [14,16]. As a whole, toxigenic isolates were recovered more commonly from patient care areas and areas proximal to patient beds, perhaps because these are the highest “traffic” area among patients and healthcare providers. Toxigenic isolates were also more likely to be recovered from restrooms and nursing stations, similar to the findings of Dubberke et al. and Dumford et al. [28,29]. Under-recognition of CDI in these two hospitals likely led to suboptimal infection control. Further research is needed to determine the role of hospital environment contamination in CDI acquisition at these two hospitals.

Ribotype distribution was similar between clinical stool isolates and hospital environmental isolates with the exception that ribotype 106 was more common in hospital environmental isolates. The most common ribotype in this study, 017, is commonly found in Asia but rarely found in the United States [16,30,31]. However, other ribotypes found in this study such as 053–163, 106, and 014–020 are ribotypes found in the United States and Europe, with the latter two being more common than the first [31,32].

This study has limitations. We collected patient and environmental samples from two large hospitals in Dhaka, Bangladesh over a defined time period. This results should ideally be replicated in a larger sample of hospitals in the country on an ongoing basis. We specifically chose traditional risk factors for CDI to assess patient risk; namely patients who received recent antibiotics with diarrhea. Whether novel risk factors for CDI are present in

Bangladesh will require further study. Likewise, future outcomes studies investigating the outcomes of patients who are under-treated for CDI are needed. Finally, we did not observe a clonal strain of CDI but rather a number of ribotypes commonly observed worldwide, including 017 that is commonly found in Asia and 106 which is common in the United States and Europe but rarely found in Asia. Further work using whole genome sequencing will be required to assess these strains in association with the global population.

5. Conclusions

Just like in resource-rich settings, “seek and you shall find” CDI in resource-limited settings in which broad-spectrum, high-risk antibiotics are used [10]. This study is the first to demonstrate that the epidemiology of CDI in at risk hospitalized patients in two major medical centers in Dhaka, Bangladesh, is similar to the epidemiology of CDI in resource-rich and other resource-limited countries. Increased awareness of CDI in resource-limiting settings is needed to increase clinical suspicion of CDI among clinicians, optimize infection control measures within local context, and regulate antimicrobial access and use in these settings.

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Fig. 1.
Hospital environmental contamination of toxigenic *C. difficile*.

Fig. 2. Ribotype distribution of clinical versus hospital environmental toxigenic *C. difficile* isolates. FP: designates a novel ribotype not present in our ribotyping library.

Table 1

Patient demographic data on day of stool sample collection.

Variable	Stool without toxigenic <i>C. difficile</i> (n = 190)	Stool with toxigenic <i>C. difficile</i> (n = 18)	p
Female sex, n (%)	77 (41)	10 (57)	0.217
Age, years, median (IQR)	45 (32–58)	39 (25–51)	0.222
Hospital			
BSMMU ^a , n (%)	151 (79.5)	14 (78)	0.770
DMCH ^b , n (%)	39 (20.5)	4 (22)	
Length of prior hospital stay, days, median (IQR)	13 (7–23)	13 (7–26)	0.990
Duration of diarrhea, days, median (IQR)	2 (2–3)	3 (2–3)	0.704
Duration of previous antibiotics, days, median (IQR)	10 (7–17)	11 (5–20)	0.837
Number of patients in the same open ward, median (IQR)	13 (7–19)	19 (10–20)	0.063
Patient was receiving metronidazole, n (%)	52 (27)	4 (22)	0.785

^aBSMMU = Bangabandhu Sheikh Mujib Medical University.

^bDMCH = Dhaka Medical College and Hospital.