ORIGINAL ARTICLE

Clostridium difficile infections among adults and children in Mwanza/Tanzania: is it an underappreciated pathogen among immunocompromised patients in sub-Saharan Africa?

M. Seugendo[1], S. E. Mshana[2], A. Hokororo[1], B. Okamo[3], M. M. Mirambo[2], L. von Müller[4], K. Gunka[5], O. Zimmermann[5] and U. Groß[5]

1) *Department of Pediatric and Child Health,* 2) *Department of Microbiology,* 3) *Department of Biochemistry and Molecular Biology, Weill Bugando School of Medicine, Mwanza, Tanzania,* 4) *Institute for Medical Microbiology and Hygiene, German Reference Laboratory for Clostridium difficile Saarland University Medical Center and* 5) *Institute of Medical Microbiology, University Medical Center Göttingen, Göttingen, Germany*

Abstract

Little is known regarding the epidemiology *Clostridium difficile* in developing countries. Fresh stool samples from patients with diarrhoea were cultured anaerobically. *C. difficile* was detected in nine (6.4%) of 141 (95% confidence interval 4.2–13.1), of which seven (77.8%) were from children. HIV infection, prolonged hospitalization and antibiotic use were independent factors associated with the occurrence of *C. difficile* in the gastrointestinal tract. Two of the toxigenic isolates were typed as ribotype 045, and the other two had unknown ribotype. All *C. difficile* isolates were susceptible to metronidazole, moxifloxacin and clarithromycin, while three isolates were resistant to clarithromycin. *C. difficile* may be an important pathogen causing diarrhoea in sub-Saharan Africa among immunocompromised patients.

Keywords: *Clostridium difficile*, diarrhoea, immunocompromised, sub-Saharan, Tanzania
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Corresponding author: S. E. Mshana, Department of Microbiology, Weill Bugando School of Medicine, Box 1464, Mwanza, Tanzania
E-mail: mshana72@yahoo.com

Introduction

Clostridium difficile is considered to be among the normal flora of the gastrointestinal tract. It mainly becomes pathogenic if the normal intestinal flora is disturbed. The use of antibiotics has been identified as the major risk factor for *C. difficile* infection in high-income countries [1]. Few studies on *C. difficile* prevalence exist from sub-Saharan Africa [2–6]; a prevalence of gastrointestinal carriage of up to 43% has been reported in one these studies. Outcome of *C. difficile* presence in gastrointestinal tract can range from asymptomatic colonization to a severe toxic megacolon with bowel perforation and sepsis [1]. In Tanzania, no studies have been performed to investigate prevalence of *C. difficile*–associated diarrhoea; therefore, for the first time we report the occurrence and associated factors of *C. difficile* among adults and children attending Sekou Toure and Bugando Medical Centre in Mwanza, Tanzania. We also report the toxigenic strain with unknown ribotype.

Methods

This was a hospital-based comparative cross-sectional study conducted between August and October 2014 at Bugando Medical Centre, a tertiary-care hospital, and Sekou Toure regional hospital in Mwanza, Tanzania. All patients with diarrhoea attending these hospitals were eligible to participate in the study. All patients with diarrhoea admitted in paediatric and medical wards during the study period were enrolled unless they failed to consent/assent. Randomly, 109 adult relatives

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without diarrhoea visiting these patients were selected and screened as control subjects for the presence of *C. difficile*. A standardized questionnaire was used to collect social demographic and clinical data, including HIV status, duration of hospitalization and history of antibiotic use and animal keeping.

Fresh stool samples from patients with and without diarrhoea were collected and processed within 4 hours. Culture was done on chromogenic agar (CHROMagar, Paris France) supplemented with CHROMagar supplements for *C. difficile*. Watery stools were plated directly, while stools from the control group were diluted with phosphate-buffered saline as previously described [7]. Plates were incubated anaerobically for 24 hours; anaerobic condition was achieved using an anaerobic gas pack (bioMérieux, Marcy l'Etoile, France). *C. difficile* isolates were tested for the glutamate dehydrogenase and *C. difficile* toxins A and B using rapid commercial tests (Quik Chek Complete, Alere Techlab, Blacksburg, VA, USA) [8]. The confirmation of the toxin production was done using multiplex PCR to detect toxin gene profile (German Reference Laboratory for C. difficile, Homburg/Saar) [9].

Susceptibility testing to metronidazole, clarithromycin, vancomycin and moxifloxacin was performed by Etest test (BMD, Marne-la-Vallée, France) following the manufacturer's guidelines, and European Committee on Antimicrobial Susceptibility Testing break points were used to interpret the results. Control strain ATCC 700057 (German Reference Laboratory for *C. difficile*, Homburg/Saar) was included to verify the reproducibility of the test. PCR ribotyping was performed according to standard protocols using capillary gel electrophoresis [10].

Data were entered in the computer using Excel (Microsoft, Redmond, WA, USA) and analysed by Stata 11 (StataCorp, College Station, TX). Continuous variables (age and duration of hospitalization) were summarized as medians, while categorical variables were summarized as proportions. The Wilcoxon rank sum (Mann-Whitney) test was performed to compare the medians of the two groups. Univariate and multivariate logistic regression analyses were done to determine the association of different factors with positive *C. difficile* culture. A p value of less 0.05 was considered to indicate a statistically significant difference.

The protocol to perform this study was approved by Bugando Medical Centre/Catholic University of Health and Allied Sciences ethics review committee, and all participants or caretakers signed informed consent.

Results

A total of 250 participants were investigated in this study. Of 250 participants; 141 (56%) had diarrhoea and 110 (44%) had no diarrhoea. Of 141 participants with diarrhoea, 69 (48.9%) were $\leq$ 12 years of age. The age of participants with diarrhoea ranged from 1 month to 65 years old, with a median age of 13 months (interquartile range (IQR) 8–60 months). Female subjects (82, 58.2%) formed the majority of participants. Most of participants without diarrhoea were adults with a mean age of 35 ± 4 years. All participants with diarrhoea were hospitalized at the time of enrollment. The median duration of hospitalization was 12 hours (IQR, 7–24 hours).

C. difficile was cultured from nine (6.4%) of 141 (95% confidence interval, 4.2–13.1) patients with diarrhoea, while none of the stool samples from 109 relatives without diarrhoea was positive. Out of seven isolates from children, six were found to be toxigenic (toxin A and B).

All nine patients who were found to carry *C. difficile* reported that they had used antibiotics previously. In addition, a total of eight (12.7%) of 63 HIV-positive individuals were found to be infected by *C. difficile* compared to only one (1.3%) of 78 among HIV-negative individuals (odds ratio, 33; 95% confidence interval, 3–370; p 0.004) (Table 1). Also, it was observed that participants from Bugando Medical Centre were significantly more infected by *C. difficile* than those from Sekou Toure (15.4% vs. 1.1%, p 0.002) (Table 1). The median duration of hospitalization of patients who were culture positive for *C. difficile* was 48 hours (IQR, 36–72 hours) compared to 12 hours (IQR, 6.5–24 hours) of those with negative stool culture for *C. difficile* (p 0.0028).

Seven isolates (four toxigenic and three nontoxigenic strains) were available for ribotyping, multiplex PCR for toxin genes and susceptibility testing. Ribotype 038 was detected in all nontoxigenic strains. Toxigenic 045 ribotype isolates (n = 2) were positive for toxin A, B and for binary toxin genes (*tcdA, tcdB*) while the two other toxigenic strains were positive for toxin A and B genes (*tcdA, tcdB*). However, PCR ribotype of both isolates remained undetermined by comparison to institutional data bank profiles. One of the two strains with unknown ribotype close similarity to ribotype 228 and 043 was noted, while the second strain showed similarity to ribotype 035 according to ribotype fragment length profiles. All seven isolates were sensitive to vancomycin, moxifloxacin and metronidazole (Table 2), with three isolates being resistant to clarithromycin.

Discussion

C. difficile in high-income countries is one of the most important causes of health care–associated infections [11–13]. In recent decades, an increase of incidence, morbidity and mortality related to *C. difficile* infection has been reported in high-income countries, such as the United States and Canada as well as

TABLE 1. Factors associated with *Clostridium difficile* infections among 141 participants with diarrhoea

	C. difficile + VE	Univariate analysis		Multivariate analysis	
Characteristic (n)	**n (%)**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Duration of hospitalization (hours)	48 (IQR, 36–72)	1.04 (1.01–1.07)	0.001	23.6 (1.2–453)	0.036
Age					
Adults (33)	3 (9.1)	1			
Children (108)	6 (5.7)	0.588 (0.13–2.5)	0.781	0.998 (0.994–1.00)	0.539
Hospital					
Sekou Toure (84)	1 (1.2)	1			
BMC (57)	8 (14.4)	13.5 (1.6–111)	0.015	10.9 (1.02–117)	0.047
HIV status					
Negative (114)	1 (0.88)	1			
Positive (27)	8 (29.6)	47 (5.62–402)	<0.001	33 (3–370)	0.004
Animal keeping					
No (93)	6 (6.4)	1			
Yes (48)	3 (6.2)	0.96 (0.23–4.4)	0.963	0.91 (0.12–6.4)	0.927

BMC, Bugando Medical Centre; CI, confidence interval; IQR, interquartile range; OR, odds ratio.

TABLE 2. Characteristics of seven isolates available for ribotyping

Strain	Subject	Ribotype	ToxA	ToxB	Binary toxin	CL	RIF	MET	MF	VA
T3	Child	Unknown	Positive	Positive	Negative	S	S	S	S	S
T91	Child	Unknown	Positive	Positive	Negative	S	S	S	S	S
T108	Adult	O38	Negative	Negative	Negative	R	R	S	S	S
T103	Adult	O38	Negative	Negative	Negative	R	S	S	S	S
T18	Child	O38	Negative	Negative	Negative	R	S	S	S	S
T15	Child	O45	Positive	Positive	Positive	S	S	S	S	S
T22	Child	O45	Positive	Positive	Positive	S	S	S	S	S

CL, clarithromycin; MET, metronidazole; MF, moxifloxacin; ND, not done; R, resistant; RIF, rifampicin; S, sensitive; VA, vancomycin.
ToxA and ToxB: *C. difficile* toxins A and B.

European countries [11,13,14]. Despite inappropriate use of antibiotics in treatment of diarrhoeal diseases in Tanzania and in other African countries [15,16], there is limited data regarding *C. difficile* infections in sub-Saharan Africa.

The prevalence rate of *C. difficile* infection/carriage in sub-Saharan Africa has been reported to range from 4% to 43% [2,3,17]. In Zimbabwe, a prevalence rate of *C. difficile* infection of 8.6% was observed [17], which is almost similar to the prevalence in this study. As observed in previous studies [14,18], no significant difference was observed between children and adults regarding infection/carriage rates; however, the high-risk group of adults aged > 65 years was missing in this study. In this study, no statistically significant difference was observed for the occurrence of *C. difficile* between children and adults [19].

It was observed that HIV-infected individuals were significantly more positive for *C. difficile* than those without HIV infection. This was previously observed in Nigeria in adults [3]. Also, we observed more carriage in malnourished children, and of two infected adults, one was HIV positive and the other had diabetes. It is therefore possible that *C. difficile* infections may be underappreciated among immunocompromised patients in Africa as a result of limited diagnostic facilities.

Another important finding in this study is detection of more cases in a tertiary-care hospital than a regional hospital. This could be explained by the fact that patients admitted to tertiary-care hospitals have received broad-spectrum antibiotic treatment for a longer time than those at regional hospitals [15]. As documented previously [1] and in this study, prolonged antibiotic use has been found to be an important risk factor for *C. difficile* infections.

The toxigenic ribotype 045 detected in this study has been detected mainly from animals such as pigs [20,21]. The detection of ribotype 045 in humans and unknown ribotype in seven isolates requires more studies to investigate the epidemiology of *C. difficile* infections in Africa.

In this study, because the aim was to detect *C. difficile* infections by culture, the toxin was assayed from colonies, not from stool specimens; this may have underestimated the magnitude of participants with positive *C. difficile* toxins. In addition, other enteric pathogens were not investigated; therefore, it is difficult for us to conclude whether *C. difficile* was the cause of diarrhoea, especially in children.

C. difficile may be an underappreciated pathogen causing diarrhoea among HIV-infected children on prolonging antibiotic treatment in Africa. Improvements in clinical microbiology services in Africa are needed to be able to diagnose *C. difficile* infections. Also, a large study to investigate the epidemiology of *C. difficile* in Africa is warranted on the basis of the fact that its epidemiology may not be the same as in developed countries.

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Conflict of Interest

None declared.

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