

***Clostridium difficile* associated diarrhoea HIV and CD4 count in Malawi**

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Abstract:

Clostridium difficile associated diarrhoea (CDAD) is the cause of substantial mortality and morbidity in the developed world. However, few data are available from the developing world, particularly in association with HIV positivity and degree of immunosuppression. We investigated the prevalence of CDAD, assessing association with HIV and CD4 count, in medical in-patients in Malawi. In 206 patients tested for CDT 28 (14%) were positive and 165 presented with diarrhoea. No significant associations were seen with either diarrhoea or advancing HIV.

The frequency and the clinical implications of CDAD in HIV positive and negative patients in SSA require further assessment.

Key Words: *Clostridium difficile*, HIV, Sub-Saharan Africa, diarrhoea

Introduction:

Clostridium difficile, a Gram-positive anaerobic spore-forming bacterium, is associated with chronic, persistent and recurrent diarrhoea leading to significant mortality and morbidity. It is currently the most common nosocomial infection in the developed world but there are few data from resource-poor settings and few studies have examined the frequency of *C. difficile* associated diarrhoea (CDAD) in sub-Saharan Africa (SSA). Published prevalence rates vary from 0% in Zambia (Kelly et al 1996) and 0.5% in the Central African Republic (Germani et al., 1998) to 7.1% in children and adults in South Africa (Samie et al, 2008) and 43% in Nigeria. (Onwueme et al, 2011). CDAD has been described as being commonly associated

with HIV in the developed world (Bartlett, 2007), but in SSA, only the Nigerian study found an association between HIV and CDAD (Onwueme et al).

We examined the prevalence of *Clostridium difficile* toxin (CDT) in the faeces of adult inpatients in a Malawian hospital. We looked for possible associations of CDT positivity with self-reported prior antibiotic use, the presence of diarrhoea, HIV status and degree of immunosuppression.

Materials and Methods:

Through 2004 and 2005, at Queen Elizabeth Central Hospital, Blantyre, Malawi, adults were recruited as part of a prospective case-controlled study on the clinical presentation and aetiology of diarrhoea in HIV positive and negative medical inpatients (MDS study). Of the 471 patients approached, 398 (84.5 %) were recruited and provided clinical details including self-reported use of antimicrobial agents prior to hospital admission. Diarrhoea was defined as the passage of three or more loose or liquid stools per day (www.who.int). HIV testing was performed using both 'Rapid' HIV ELISA tests and UniGold™ (Trinity Biotech, Wicklow, Ireland) and Determine™ H (Abbott Laboratories, Abbott Park, IL, USA). In line with National Aids Commission guidelines (www.aidsmalawi.org.mw), HIV infection was confirmed if two tests were positive and absent if both tests were negative; if test results were discordant, a third rapid HIV antibody test, Med Mira Rapid HIV Test™ (MedMira Laboratories, Halifax, Canada) was performed as a tie-breaker. HIV positive patients were offered a CD4 cell count, carried out at the Malawi-Liverpool Wellcome Trust (Trucount™, Becton Dickinson, UK). CD4 counts were grouped as less than 50 cells/mm³, less than 200 cells/mm³, and greater than 200 cells/mm³.

206 stored stool samples were subsequently available for testing for the presence of CDT. Faecal samples were stored in a cryotube immediately after collection and frozen and stored at -80°C until shipment back to the Department of Medical Microbiology, University of Liverpool, UK. Samples were then defrosted and tested for presence of CDT toxin using a validated single-step enzyme immunoassay, according to the manufacturer's instructions (TECHLAB® Tox A/B Elisa Test; www.techlab.com.) All data were entered into a secure, anonymised database and analysed using EPI-Info 3.3.2 and SPSS version 14. Comparisons between groups were performed using Fisher's exact test. All patients gave written informed consent to participate in the study, which was approved by the College of Medicine research and ethics committee. .

Results:

Overall, 28/206 (13.6%) faecal samples were positive for CDT toxin. 165/206 (80%) of patients presented with diarrhoea (either acute or chronic in nature), of whom 22 (13.3%) tested positive for CDT toxin, compared to 6/35 without diarrhoea (NS). 21 (75%) of the CDT positive patients reported that they had purchased or used some form of antibiotic (the identity of which was frequently unknown) in the last month. Of the 28 CDT positive patients, 21 (75%) were HIV positive, compared to 153/17 (86%) of CDT negative patients (p=0.16). Within the HIV positive group, there was no associations between presence of CDT and CD4 level. (**Table 1**).

Discussion:

This is the first study in Malawian adults to describe the presence of CDT toxin in stool samples. It is also the first in sub-Saharan Africa to examine possible associations of CDT with advancing HIV using CD4 counts. No associations were seen with diarrhoea and either HIV positivity or CD4 count, although significance for CD4<50 cells mm³ was almost reached (p=0.058). This is contrast to developed world literature (Bartlett,2007, Collini PJ et al, 2012), and requires further evaluation.

Mortality associated with CDAD is greater than that from all other intestinal infectious diseases combined in the United States. (www.cdc.gov/HAI/organisms/cdiff), but its impact has not been established in the developing world setting. Risk factors for CDT, in the Western setting, include antibiotic exposure, age, other co-morbidities and hospitalization. Further evaluation is needed in the resource poor.

Limitations of the study include a lack of detail about the types of antibiotic used by patients, the relative small size and possible inaccuracy of toxin testing with freezing and de-frosting.

The limited sensitivity of a single ELISA in detecting CDT is well recognised, but should not affect comparison between groups within the study.

Conclusions

Despite the methodological limitations of this study, we believe it provides important findings. It is one of the few to examine CDT in SSA, yielding a prevalence of 14%. This falls somewhere between the low and nil prevalence in central and southern Africa and the very high rates in Nigeria.

Despite assertions in the literature regarding the associations between CDT and HIV, based on a very limited evidence base, we found no association with HIV or degree of immunosuppression, contradicting the Nigerian findings.

We would propose that there is a need for further randomised control studies in SSA, preferably incorporating culture and ribotyping of positive samples.

Authors contributions:

MBJ Study design and conduct, statistical analysis and manuscript preparation.

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TL Assisted in study conduct.

EZ Study design and manuscript preparation.

AW Study design and manuscript preparation.

BF Study design and statistical analysis.

LW Assisted in manuscript preparation.

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Conflicts of interest: None declared.

Ethical approval: Ethical approval was granted by the University of Malawi College of medicine ethics committee. (COMREC)

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Table 1. detailing association between presentation, CDT result, HIV status and CD4 count

	Overall		p-value	Overall Diarrhoea		p-value	Acute Diarrhoea		p-value	Controls (non diarrhoea)		p-value
	CDT Positive n= 28	CDT Negative n= 178		CDT Positive n=22	CDT Negative n=143		CDT Positive n=11	CDT Negative n=59		CDT Positive n=6	CDT Negative n=35	
HIV Negative	7	25	0.16	5 (23%)	14 (10%)	0.141	5 (45%)	11 (19%)	0.110	2 (33%)	11 (31%)	1.00
HIV Positive	21	153		17 (77%)	129 (90%)		6 (55%)	48 (81%)		4 (67%)	24 (69%)	
CD4 <50	13	59	0.058	Key: CDT: <i>Clostridium Difficile</i> Toxin; HIV: Human Immunodeficiency Virus; CD4:								
CD4 >50	7	82										
CD4<200	17	116	1.00									
CD4>200	3	25										

