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Clostridium difficile Toxin in Adult Inpatients in an Urban Hospital in Malawi: Associations with HIV Status, CD4 Count and Diarrhoea

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Abstract: *Clostridium difficile* Infection (CDI) is the cause of substantial morbidity and mortality in the developed world. However, very little is known about the burden of CDI in sub-Saharan Africa where less antibiotic restriction, high HIV prevalence and greater impact from nosocomial infection mean the potential for a significant disease burden is great. Researchers investigated the prevalence of *Clostridium difficile* Toxin (CDT), assessing association with HIV, CD4 count and diarrhoea in medical in-patients in Malawi. In 206 patients tested for CDT, 28 (13.6%) were positive. No significant associations were seen with either diarrhoea or HIV. There was a non-statistically significant ($p = 0.056$) association between CD4 counts of <50 and CDT. The frequency and the clinical implications of CDI in both HIV positive and negative patients in sub-Saharan Africa, requires further assessment.

Key words: *Clostridum difficile*, HIV, sub-Saharan Africa, diarrhoea, CDI

INTRODUCTION

Clostridium difficile is a gram-negative spore forming bacterium. The spectrum of *Clostridium difficile* Infection (CDI) ranges from asymptomatic infection to severe life threatening colitis with a significant morbidity and mortality. In developed healthcare systems it is a major cause of nosocomial infection with an observed increase in incidence and virulence in recent years (Kachrimanidou and Malisiovas, 2011). Developed healthcare systems have therefore dedicated significant resources into the research, prevention and treatment of CDI. The primary risk factor in this setting is antibiotic usage and CDI is known to cause 15-25% of all antibiotic associated diarrhoea (Bartlett, 2007). It is also known that CDI is described as a leading cause of bacterial diarrhoea in HIV positive individuals in the developed world. However, very little is known about the disease burden of CDI in sub-Saharan Africa. Published rates of CDI vary from 0% in Kenya to 43% in Nigeria (Mwachari *et al*., 2011; Onwueme *et al*., 2011). There is also a minimal understanding of the link between HIV and CDI in this area of the world. There are currently no published data on the association between CDI and CD4 count in HIV positive patients. Table 1 summarises the literature published on *Clostridium difficile* prevalence and its association with HIV in different sub-Saharan countries.

The aim of this study was to describe the prevalence of *Clostridium difficile* Toxin (CDT) in adult inpatients in an urban hospital in Malawi and to explore the association between the presence of diarrhoea, the patient's HIV status and the degree of immunosupression. It is the first study of CDI in Malawi and the first to assess the relationship with CD4 count in sub-Saharan Africa.

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

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Table 1: Published rates of CDI in sub-Saharan Africa and association with HIV

Researchers	Countries	Sample size	Prevalence (%)	Diagnostic test for *Clostridium difficile*	HIV association
Mwachari *et al.* (2011)	Kenya	75	0.0	Cytotoxicity assay	N/A
Germani *et al.* (1998)	Central African Republic	430	0.7	Cytotoxicity assay	N/A
Samie *et al.* (2008)	South Africa	322	7.1	PCR for cytotoxin genes	No
Onwuema *et al.* (2011)	Nigeria	140	4.3-43.5	EIA for toxin A and B	Yes
Rajabally *et al.* (2013)	South Africa	643	9.2	EIA for toxin A	No

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 <50 cells mm^{-3} <200 cells mm^{-3} and >200 cells mm^{-3}.

The 206 stored stool samples were subsequently available for testing for the presence of CDT. Of these, 165 (80%) were suffering with diarrhoea at the time of sample collection. 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. The 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 (17.1%) without diarrhoea. Of the 174 patients who were HIV positive, 21 were CDT positive (12.1%) versus 7/32 (21.1%) who were HIV negative (p = 0.16). In patients with known CD4 counts, in those with CD4 count <50, 13 were positive for CDT (19.1%) versus 7 (7.9%) with CD4 count >50 (p = 0.058). In those with CD4 count <200, 17/133 (12.8%) were CDT positive versus 3/28 (10.7%) with CD4 count >200 (p = 1.00) (Table 2 and 3).

Table 2: Associations between CDT result, HIV status and presence of diarrhoea

	Overall (n = 206)		
Sub-group	CDT positive (%)	CDT negative (%)	p-values
Total cohort (n = 206)	28 (13.6)	178 (86.4)	-
HIV+ve (n = 1740)	21 (12.1)	153 (87.9)	0.16
HIV-ve (n = 32)	7 (21.9)	25 (78.1)	-
Diarrhoea (n = 165)	22 (13.3)	143 (86.7)	1.00
No diarrhoea (n = 41)	6 (14.6)	35 (86.4)	-

Table 3: Associations between CDT result and CD4 count

	Overall patients with known CD4 count (n = 161)		
CD4 sub-group	CDT positive (%)	CDT negative (%)	p-values
CD4<50	13 (18.1)	59 (81.9)	0.058
CD4>50	7 (7.9)	82 (92.1)	-
CD4<200	17 (12.8)	116 (87.2)	1.000
CD4>200	3 (10.7)	25 (89.3)	-

DISCUSSION

The results of the study suggest that CDT is prevalent in Malawi and that its presence is not associated with HIV. This is consistent with the majority of published studies from sub-Saharah Africa and differs from the developed world (Bartlett, 2007; Collini *et al.*, 2012). The results suggested that there may be an increased carriage of CDT with severe immunosuppression (CD4 counts <50) in Malawi but this association did not reach statistical significance and requires further characterisation. CDI as a nosocomial infection represents a major cause of morbidity and mortality in developed healthcare systems where the primary risk factor is antibiotic usage. However, it remains poorly characterised in sub-Saharah Africa. This gap in understanding is particularly important given the widespread availability of antibiotics in many sub-Saharan countries and less restriction on their use (Becker *et al.*, 2002). It has also been shown that developing countries have a significant burden of nosocomial infection with an

even greater impact than in developed countries (Allegranzi *et al*., 2011). With this in mind, there is a clear potential for CDI to contribute to the burden of disease within the developing world warranting further research.

Interestingly, in the study the rates of CDT in stool were no different between the diarrhoeal group and the non-diarrhoeal group. This contrasts with the most robust study of CDI in sub-Saharan Africa where an association with toxinogenic *C. difficile* and diarrhoea was found and it was concluded that CDI contributed to the diarrhoeal disease burden in South Africa (Samie *et al*., 2008). It is also well known that there is a significant rate of carriage of *C. difficile* in asymptomatic patients in developed healthcare systems with rates varying widely (Ozaki *et al*., 2004). Asymptomatic carriage of *C. difficile* contributes to the problem of disease control in these healthcare systems (Riggs *et al*., 2007). The significance of *C. difficile* carriage in sub-Saharan Africa is not yet established.

CONCLUSION

It is important to recognise that CDT is present in the study in Malawi. It is not associated with HIV infection, diarrhoea nor significantly with CD4 count in the study. In resource poor settings in sub-Sarahan Africa with less antibiotic restriction antibiotic usage and limited infection control measures, the burden of CDI remains poorly understood and warrants further investigation.

LIMITATIONS

Limitations of the study include a lack of detail about the types of antibiotic used by patients and possible inaccuracy of toxin testing with freezing and de-frosting. CDT's immunological activity is known not to be affected by freezing to -20°C yet the effect of freezing to -80°C is not known. This may lead to false negative CDT results but is unlikely to cause false positive results. The limited sensitivity of a single ELISA in detecting CDT is well recognised but should not affect comparison between groups within the study.

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