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Short Communication

High prevalence of toxinogenic *Clostridium difficile* in Nigerian adult HIV patients

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ABSTRACT

Clostridium difficile is the most commonly identified bacterial cause of nosocomial and HIV-related diarrhea. In many developing countries, antibiotic access is unregulated. Nigeria has the third highest HIV burden worldwide. Due to perceptions of low prevalence and resource incapacity, patients with diarrhea are not tested for toxinogenic *C. difficile* infection (CDI). In this pilot study which included 97 HIV-positive patients at two hospitals in Nigeria, the estimated prevalence of CDI was 43% and 14% for in-patients and out-patients respectively. HIV-positive out-patients were more likely to have toxinogenic CDI than non-HIV out-patients ($P=0.007$, Fisher's exact test).

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1. Introduction

Clostridium difficile is the most commonly identified bacterial cause of HIV-related and all nosocomial diarrhea.¹ Whereas outbreaks in North American and European hospitals have led to increased *C. difficile* infection (CDI) vigilance in developed countries, data on its significance for developing countries is scarce.² This information gap is significant since antibiotic pre-exposure, an important CDI risk factor, is high in many developing countries due to unregulated access.³ Recent data suggest CDI is under-recognized in Latin America.^{4,5} Data from sub-Saharan Africa is sparse.

Nigeria, Africa's most populous country, has the third highest absolute HIV burden worldwide. Over-the-counter antibiotic access is widespread in Nigeria and nosocomial diarrhea incidence is high.⁶ However, patients are not tested for CDI due to presumptions of low prevalence and technical incapacity. We hypothesized that many cases of nosocomial and community-acquired diarrhea in Nigeria may have a CDI etiology that is under-recognized due to lack of surveillance. To explore this we conducted a pilot study to estimate toxinogenic CDI prevalence in Nigerian HIV patients. A secondary aim was to demonstrate that CDI surveillance is within extant resource capacity of Nigeria's HIV treatment centers.

2. Methods

Patient stool specimens were collected between October 2008 and August 2009 at two urban hospitals in Nigeria: Jos University Teaching Hospital (JUTH), a 500

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bed public hospital and its HIV Clinic, and Zankli Medical Center (Zankli), a 55 bed hospital in Abuja.

JUTH in-patients had been hospitalized for greater than 24 hours and reported greater than two witnessed watery bowel movements/day for at least 48 hours. For out-patients diarrhea was defined as reported loose stools occurring greater than twice a patient's regular frequency. Zankli patients endorsing at least a two day history of loose stools were recruited during routine triage regardless of diagnosis.

Informed consent, symptom and risk history were obtained via an English language form translated when necessary by a healthcare worker. Ethical approval was obtained at each institution.

Same day stool specimens or fresh-frozen specimens stored for less than seven days at -20°C without freeze-thaw cycles were assessed for *C. difficile* toxin A or B using rapid enzyme immunoassay (EIA) (Inverness Medical Professional Diagnostics, Princeton, NJ, USA). Assays were analyzed visually and/or spectrophotometrically (OD 450 nm/650 nm >0.05). Providers were notified of results.

3. Results

One hundred and forty adult patients (94 from JUTH and 46 from Zankli) participated in the study; 97 were HIV-positive and 43 HIV negative. Seventy-one JUTH patients were out-patients and 23 were in-patients. All 94 JUTH patients and three Zankli patients were HIV-positive.

Excluding uncomplicated pulmonary tuberculosis, no JUTH out-patients had known AIDS complications, all were on anti-retrovirals (ARVs) and none were from the same household. Patients did not recall exposure to sick contacts with diarrhea, non-prescription antibiotic use or hospitalization within the preceding three months. Of the 71 out-patients 10 (14%) had toxinogenic CDI (Table 1).

In contrast all 23 JUTH in-patients had been admitted for AIDS-related complications (e.g. miliary tuberculosis,

Kaposi's sarcoma and bacterial peritonitis). Of this group, 10/23 (43.5%) had toxin-positive CDI (Table 1). Six toxin-positive and 8/13 toxin-negative in-patients were on ARVs. There was no discernible ward-clustering as the positive samples came from six geographically distinct wards. While over-the-counter antibiotic use was common, timing was not reliably recalled by patients.

Principal diagnoses for Zankli patients varied but none were admitted specifically for the incidental bowel habit changes documented on systems review. Three patients were HIV-positive and the remaining 43 with no known HIV history were considered negative. Twenty-one Zankli patients reported antibiotic use within the preceding month. Metronidazole was the most common agent of choice (not shown). The only toxin-positive sample from Zankli came from an HIV-positive patient on ARVs (Table 1).

4. Discussion

Nigeria is a representative developing country with a high HIV burden. Our findings indicate that CDI is prevalent in Nigeria's HIV population including those on ARVs. The suggested prevalence of 14% and 43% for symptomatic HIV-positive out-patients and in-patients respectively is startling and recapitulates emerging observations from Southern Africa.^{7,8} To our knowledge, we provide the first data on CDI prevalence in West-African HIV patients.

The association between CDI and HIV in out-patients was statistically significant ($P=0.007$, Table 1). However, the small size of this pilot study limits interpretation. Also, although toxin EIA has the advantage of rapid result-ing, it was recently shown to be less sensitive than once believed so that our findings may underestimate true prevalence in this population.^{9,10} Ready reagent availability and rapid turn-around however make CDI toxin-EIA suited for resource-constrained settings.

Barriers to routine CDI testing in Nigeria include limited capacity, perceptions of low prevalence and cost. Newer assay modalities are bridging materials-related incapacity. Many facilities have extant materials and recruitable human-resource capacity as we demonstrate. While larger studies are required, it is clear that, in places like Nigeria, guidelines are needed for symptom-triggered CDI testing in high-risk populations.

Authors' contributions: CU and KO conceived the study. CU, KO, JI and LL designed the study; all authors critically reviewed and approved the study proposal; LI, JO, OA, KO and YF collected data; KO, YF, CU and PA analyzed and interpreted the data; KO drafted the manuscript; all authors read and critically reviewed the manuscript for intellectual content; all authors approved the final version of the manuscript. KO and CU are guarantors of the paper.

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Table 1

Prevalence of toxinogenic *Clostridium difficile* infection in Nigerian patients

	JUTH (out-patient) ^a	JUTH (in-patient)	ZANKLI (out-patient) ^a
Total	71	23	46
HIV	71	23	3
ARV	71	14	3
Pre-exposure to non-tuberculosis antibiotic	0	$\geq 3^b$	21
<i>C. difficile</i> Toxin positive	10	10(6 on ARV)	1
<i>C. difficile</i> Toxin negative	61	13(8 on ARV)	45

JUTH: Jos University Teaching Hospital; ZANKLI: Zankli Medical Center; ARV: Anti-retrovirals.

^a For out-patients, the association of toxinogenic CDI with HIV was statistically significant ($P=0.007$, Fisher's Exact test; contingency table data: HIV positive + toxin positive = 11; HIV positive + toxin negative = 63; HIV negative + toxin positive = 0; HIV negative + toxin negative = 43).

^b 3 patients recalled specific timing and nature of pre-hospital antibiotic exposure. The remainder either could not recall pre-hospitalization antibiotic use or time of use. All JUTH in-patients were on antibiotics for opportunistic AIDS infections at the time of stool collection.

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Conflicts of interest: None declared

Ethical Approval: Ethical approval for this study was obtained at the institutional review boards of Jos University Teaching Hospital, Nigeria, Zankli Medical Center, Nigeria and Partners Healthcare (for Brigham and Women's Hospital), Boston USA.

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