

## Environmental Sources *Clostridium difficile* in Lagos State, Nigeria

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### Abstract

*Clostridium difficile* is the pathogen responsible for antibiotic-associated pseudo-membrane colitis (diarrhea), which can be as a result of the use of broad spectrum antibiotics (such as ampicillin, clindamycin and the cephalosporins). These antibiotics can wipe away part of the normal intestinal flora allowing the pathogenic *Clostridium difficile* that is sometimes present to super infect the colon. When it grows in abundance, it releases its exotoxins. Toxin A causes diarrhea, and Toxin B is cytotoxic to the colonic cells. *Clostridium difficile* is known to cause severe diarrhea, abdominal cramping and fever. In this study, a total of 480 environmental samples were collected. Soil (100), Water (100), Vegetables (100), Animal Faeces (100) and the Hospital environment (80). Using Brazier CCEY Agar, *Clostridium difficile* spores were only found in the hospital setting examined. Seven (7) out of the Sixty (60) samples examined from the Medical Wards as well as three (3) out of the twenty (20) Intensive Care Unit (ICU) samples were *C. difficile* positive. In the medical wards, the areas examined are the patient bed sheet, bed railings, table top, toilets and windows. From the ICU, the spores were found on the drip stand, table tops and patient beds. *Clostridium difficile* was not isolated from Soil, Water, Vegetables and Animal Faeces but other species was isolated from them. This suggests that *C. difficile* is more easily isolated in the hospital environment than the community. Therefore, more samples are still needed to be examined in more locations and other sources within the hospital as well as the community so as to improve our understanding about the ecology of *C. difficile* transmission.

### INTRODUCTION

*Clostridium difficile* was once a relatively obscure organism to the general public but has recently gained prominence as a highly virulent intestinal microorganism capable of causing protracted hospital-associated diarrheal illnesses, especially in situations when antibiotics are administered (Taubes, 2008). Contributing factors to this greater awareness are a corresponding increase in the incidence of *C. difficile* infections (CDI), heightened severity of disease symptoms with higher reoccurrence rates, and an increase in antimicrobial resistance particularly in the United States (Wiegand *et al.*, 2012).

The increase in CDI cases has spurred increased concern regarding the growing segment of community-associated infections distinct from infections acquired in healthcare settings (Limbago *et al.*, 2009).

Importantly, some evidence has shown that *C. difficile* may be brought into the healthcare environment by asymptomatic carriers (Clabots *et al.*, 1992). The reported carriage rates of *C. difficile* in healthy adults have varied from 0% to 3% in Europe to up to 15% in Japan (Mulligan, 2008).

However, little is known about the prevalence of *C. difficile* in the environment and how it may be transmitted to humans. In some environments, *C. difficile* has been found in a variety of environments, including water, soil, animal faeces, and foods (Al Saif and Brazier, 1996; Rodriguez-Palacios *et al.*, 2007). These findings suggest that *C. difficile* may be transmitted to humans through food, although no foodborne cases have been reported.

In previous studies, ready-to-eat foods have been implicated in foodborne disease outbreaks associated with *Salmonella* species and *Escherichia coli* O157 (Sagoo *et al.*, 2003 and Delaquis *et al.*, 2007)

In Nigeria, the distribution of *C. difficile* in the general environment is largely unknown. This study is aimed at investigating how healthy individuals in the community may be exposed to *C. difficile* and also to promote an understanding of the ecology of the organism. The aim of this present study is to determine the potential sources of *Clostridium difficile* in Lagos environment

Several studies have reported the widespread presence of the organism in hospital wards and on the hands of nursing personnel (Macfarland *et al.*, 1989). However, fewer studies have been directed at the environment outside the hospitals. Reports of *C. difficile* in the environment suggest either that its geographical distribution is not known or that different methodologies used were responsible for different isolation rates. (Al-Saif and Brazier, 1996)

For example, Hafiz from Sheffield, England in 1976 reported the organism in soil, sand and mud. Other workers in Korea and Poland have also found it in the soil. However, a study in Perth, Western Australia and a Study of 20 random soil samples from Michigan, USA both gave negative results. (Kim *et al.*, 1981)

Furthermore, in a broad study carried out by Al-Saif and Brazier in 1996, only 184 out of 2580 samples representing about 7.1% yielded positive isolates from the domestic environment in Cardiff area of South Wales. Kim (1981) reported positive cultures from 12.2% of samples from floors and random surfaces in several rooms

within a house occupied by a case of *C. difficile* diarrhoea. As a control, the home of healthy person was also sampled and no positive cultures were reported.

## **MATERIALS AND METHODS**

### **STUDY AREA**

Water, Soil, Farm Animal Faeces and Raw Vegetables were collected from Five (5) Local Government Areas of Lagos State from the 27<sup>th</sup>-31<sup>st</sup> of August, 2012 using a simple stratified random sampling method.

### **CULTURE AND IDENTIFICATION**

#### **Processing of Water Samples**

One Hundred (100)  $\mu$ l of the water was filtered through 0.45 $\mu$ m filter membrane. The membrane filter was aseptically placed on the prepared Brazier's Selective Medium and incubated anaerobically at 37°C for 48hrs in Anaerobic Jar (Biomerieux, France) in N<sub>2</sub>(80%), H<sub>2</sub>(10) and C<sub>o</sub>2 (10%). Anaerobiosis was achieved using Anaerobic Indicator which changes from Blue to White in the presence of *Pseudomonas aeruginosa* ATCC 27853. All culture plates were cultured for 48hrs and plates showing no growth were re-incubated for another 24hrs before being discarded. All isolates grown were tested with Metronidazole antibiotic disc. The Isolates that shows Gram positive rods with spores were presumptively identified as Clostridia and stored in 15% Anaerobic Glycerol broth in -80°C for further identification. (Al Saif and Brazier, 1996)

#### **Processing of Soil/Animal Faeces**

All soil and animal faecal samples were mixed vigorously in the same ratio with 15% methyl alcohol and left to stand for 30 minutes to knock-out all vegetative cells leaving only the spores. The mixtures was then allowed to settle and a loopful of the deposit was aseptically streaked on already prepared Brazier CCEY medium and incubated anaerobically at 37°C for 48hrs in Anaerobic Jar (Biomerieux, France) in N<sub>2</sub>(80%), H<sub>2</sub>(10) and C<sub>o</sub>2 (10%). All plates showing no growth were further re-incubated for another 24 hrs before being discarded. (Al Saif and Brazier, 1996)

#### **Processing of Vegetables**

The unwashed surfaces of the samples was placed directly on the medium and then discarded. Vegetables samples are Ugwu Leaves, Ewedu, Efo tete, Onions and Tomato. Anaerobic incubation was carried out as described for the above samples. (Al Saif and Brazier, 1996)

### **BIOCHEMICAL IDENTIFICATION**

Biochemical identification of *C. difficile* was carried out using API 20A (BioMerieux) Identification System. The system enables 21 tests to be carried out quickly and easily for the biochemical identification of anaerobes. Other test such as colonial and microscopic morphology, Gram stain was performed and the results used to complete the identification.

## **RESULT**

A total of 480 samples were collected for this study from the environment. These include Soil (100), Water (100), Vegetables (100), Animal Faeces (100) and the Hospital environment (80). *Clostridium difficile* spores were only found in the hospital setting examined. Seven (7) out of the Sixty (60) samples examined from the Medical Wards as well as three (3) out of the twenty (20) Intensive Care Unit (ICU) samples were *C. difficile* positive. In the medical wards, the areas examined are the patient bed sheet, bed railings, table top, toilets and windows. From the ICU, the spores were found on the drip stand, hospital floors, table tops and patient beds. It was observed from the medical records that patients in both sections of the hospital (Medical wards and ICU) are on heavy antibiotic use. Four (4) out of the Five (5) patients seen in the ICU were on post-surgery treatment while one (1) was reported to be recovering from complications arising from a caesarian operation as a result of prolonged labor. The patient was also observed to be passing loose stool; an indication of a possible diarrheal infection.

In the medical wards, (A3), a patient was seen passing stool while some others were seen passing urine in plastic containers brought by the health attendant who later disposed it in the toilet. This can be a potential way in which *Clostridium difficile* spores may escape into the surrounding environment if it had already colonized the gut of the patients.

The result from the hospital setting was not unexpected as *C. difficile* has been demonstrated to cause a health-care associated infection.

There were no *C. difficile* spores in the Soil, Water, Vegetables and Animal Faeces but other *Clostridia* species were found in them.

Table 1 shows the summary of results obtained for the Identification of *Clostridia* species from Environmental Samples.

In the Soil, Water, Animal faeces and Vegetables samples examined, no *C. difficile* was identified but other

Clostridia species were found in some of the samples.

**Table 1: Summary results for the Identification of Clostridia species from Environmental Samples**

Source	n	Local Government Areas	Number(%) samples positive
Soil	100	Agege (10), Ojo (9), Epe (10)	29(29)
Animal Faeces	100	Epe(20), Agege (10), Epe (10) Lagos Mainland (20)	60 (60)
Water Samples	100	Epe(11) Oshodi (10) Agege (10)	31(31)
Vegetables	100	Epe (20),Agege (20), Ojo (20), Oshodi (20) &Lagos Mainland (20)	0(0)
Hospital Environment		Medical Wards & ICU	10 (12.5)
<b>Total</b>	<b>480</b>		<b>130(27.1)</b>

N: number of samples

## DISCUSSION

This study investigated the presence of *C. difficile* spores in the environment. It was observed that *C. difficile* spores were only found in the hospital environments (the medical wards and intensive care unit) but were not found in the environmental samples examined (water, soil, animal faeces, and vegetables).

7 out of the 60 samples examined from the medical wards from the patient bed, floors, toilet and windows. Record shows that the patients were all above 50 years of age, on heavy antibiotic use while others have stayed in the hospital for about 1-2 months. Some of the patients were seen passing stool and urine in a plastic container provided by the hospital health attendant before being disposed in the toilet. This can be a means of *C. difficile* spore transmission to the surrounding environment if the patient has been colonized with *C. difficile* spores.

In the Lagos University Teaching Hospital-Intensive Care Unit (LUTH-ICU), 3 out of the 20 samples examined from the drip stand, bed rails and table top was identified as *C. difficile* positive. At the time of examination, record shows that 4 out of the 5 patients have been admitted for about 7 weeks, on heavy drug administration and above 55 years of age. These are some of the identified risk factors for *C. difficile* colonization in the hospital.

One of the patients had medical complications arising from Caesarian Section of her womb after prolonged labour. The patient was reported to be passing loose stool. This may be an indication that she had diarrhea.

The result obtained in this research from the hospital setting was not unexpected as *C. difficile* has been demonstrated to cause a health-care associated infection.

Several studies have reported the widespread presence of *C. difficile* in hospital wards and on the hands of nursing personnel (Kim *et al.*, 1981; Macfarland *et al.*, 1989; Egwuatu, 2011, unpubl).

Macfarland *et al.*, (1989) reported that the study of the epidemiology of *C. difficile* has identified both asymptomatic *C. difficile* culture-positive patients and contaminated environments as potential sources of diarrheal outbreaks. Some other studies have reported the environment surrounding both symptomatic and asymptomatic patients as reservoir for *C. difficile* which is potentially transmitted by contact with fomites, staff and other patients.

Best *et al.*, (2010) informs that that though the spores of *C. difficile* can occasionally spread through the air but can be easily transmitted through the hands of hospital staff.

The isolation of 3 *C. difficile* positive culture out of the 20 (15%) samples examined in the LUTH-ICU was not unexpected. Studies conducted by Titov, *et al* (2000); Fawley and Wilcox, (2001) and Egwuatu, (2011) all confirmed and supported that in the hospital particularly the ICU, strains of *C. difficile* may be introduced by hospitalized patients or staff and transmitted to other patients and their environment.

In another study carried out in Kuwait, Rotimi and other researchers (2002) reported that the acquisition rate of *C. difficile* increased from 5.9 to 36% during 4 to 53 days of hospitalization in various wards.

From the results obtained in this study, 7 out of the 60 samples examined in the medical wards were *C. difficile* positive. A recovery rate of 12.5% from the LUTH hospital environments suggest that the main route of transmission may be through hospitalized patients. In addition, the low rate of recovery (12.5%) may be as a result of the wards sampled, the underlying medical conditions of the in-patients, the environment, the staff as well as the technique of sampling employed.

In a study by Conly (2000), the risk of colonization by *C. difficile* was found to increase in a direct proportion to the length of hospital stay ranging from 13% among patients admitted for less than 1 week to as high as 50% among patients admitted for more than 4 weeks; this suggests that exposure to *Clostridium difficile* occur throughout the hospital stay.

In another carried out by Akhi *et al.*, (2011), out of 70 *C. difficile* isolates which were cultured as a first time in North West Iran, 18% (18/100), 10.37% (14/135), 32% (16/50) and 44% (22/50) were isolated from staff, hospital environment, patients at first day of admission and the same patients after seven days of hospitalization respectively. Six patients (12%) were reported to be colonized by *Clostridium difficile* during days of hospitalizations.

Fourteen isolates (10.37%) of *Clostridium difficile* was obtained from various region of pulmonary (n=5), infectious disease (n=3), ICU of neurology (n=3), ICU of pulmonary (n=2) and endocrine and rheumatology (n=1) wards.

In this study, no *C. difficile* was identified from the environmental samples but were found in the hospital environment. For instance in a broad study carried out by Al-Saif and Brazier (1996) using a total of 2,580 samples, only 184(7.1%) yielded *C. difficile* from samples obtained from the hospital environment as well as some environmental samples. From their study, it was suggested that the non-isolation of *C. difficile* from the environment may be due to their geographical distribution or by the cultural methodology applied which may cause different isolation rates. This shows that the isolation of *C. difficile* in the environment may be very difficult to establish.

Furthermore, a study in Perth, Western Australia and a study of 20 random samples from Michigan, USA (Kim *et al.*, 1981; Riley 1994) both gave negative results for the isolation of *C. difficile*. All these further confirm the result obtained in this present study.

The results of this study also suggest that the consumption of Vegetables is not likely to be an important source of exposure to the bacterium. In addition, other samples such as water, animal faeces and soil appear seem to have no or low risk potential as no *C. difficile* was identified from them.

The presence of other *Clostridia* species in water samples has been demonstrated in several studies (Davies 1969). If *C. difficile* were to be present, it shows the potential of the water supplies to be a source of infection in case of treatment failure.

*Clostridium perfringens* was isolated from the water samples examined. This finding is in agreement with previous studies which show that their presence is thought to indicate a failure in the water filtration process. The study showed that fifty (50) out of fifty-five(55) anaerobic bacilli was isolated from sand filters in sand beds to show that they could be reservoir of *Clostridia* species (Nankiveli, 1911). In Addition, it could be as a result of faecal contamination of the water source and may be a potential source of *C. difficile* in the near future.

In Nigeria, there is minimal literature concerning the transmission of *Clostridium difficile* from hospital staff to patients and none looking solely at doctors but the hands of hospital personnel caring for patients with *C. difficile* often become colonized with the bacterium thereby facilitating transmission among hospital in-patients. Therefore, more studies can still be carried out in this regard to ascertain their level of transmission.

## CONCLUSION

In the Lagos population examined, *C. difficile* was only found within the hospital setting but was not identified from the environmental samples. This suggests that *C. difficile* is more easily isolated in the hospital environments than the general environment. More samples are still needed to be taken in more locations and other sources within the hospital as well as the larger community to improve our understanding of the ecology of *C. difficile* transmission.

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