

## Prevalence of *Clostridium difficile* in Hospital Environment within Yola Adamawa State Nigeria

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### ABSTRACT

**Introduction:** *Clostridium difficile* infection (CDI) is an emerging health problem in hospital setting. The ability of the spores to persist in the environment is a key factor in rates of infection. *Clostridium difficile* has also been described as one of the leading cause of nosocomial diarrhea. It is responsible for an increase in hospital stay with high healthcare and economic repercussions.

**Objectives:** This study was aimed at determining the prevalence and antimicrobial susceptibility profile of *Clostridium difficile* isolated from surfaces within hospital environment in Yola Adamawa State Nigeria.

**Materials and Methods:** A total of 150 surface samples were collected from different wards of two hospitals (Specialist Hospital Yola (SHY) and Federal Medical Centre Yola (FMCY)) using moistened swabs. *Clostridium difficile* isolates were obtained by enriching and culturing samples in cycloserine-cefoxitin fructose broth (CCFB) and cycloserine-cefoxitin fructose agar (CCFA) respectively. Susceptibility test (MIC determination) was done by well diffusion technique on Mueller Hinton agar supplemented with 5% sodium taurocholate.

**Results:** A total of 18 (12%) *Clostridium difficile* were recovered from the two hospitals sampled. FMC had a prevalence rate of 13.3% with table top, toilet, window and bed sheets as sites implicated while SHY had a prevalence rate of 10.7% with bed sheet, bed railing and window as implicated sites. The antibiotic susceptibility test reveals that *Clostridium difficile* isolates obtained were resistant to the antibiotics tested which includes Ciprofloxacin (MIC >64 µg/ml), Erythromycin (MIC >64 µg/ml), Metronidazole (MIC 64 µg/ml), Tetracycline (MIC > 128 µg/ml) and Clindamycin (MIC > 64 µg/ml).

**Conclusions:** The study depicts that multidrug resistant *Clostridium difficile* is prevalent in hospital environments within Yola Adamawa State Nigeria. The need for the sensitization of healthcare workers to improve understanding of *Clostridium difficile* transmission, treatment, management and prevention is of paramount importance.

### Keywords

*Clostridium difficile*, Diarrhea, Hospital environment, Nosocomial prevalence, Spores.

### Introduction

*Clostridium difficile* has been recognized as a cause of antibiotic-associated diarrhea since 1978 [1]. Recently as incidence and severity of cases have increased, it has become an infection that requires emergency control [2]. There are also reports of increased

mortality due to some epidemiological strains [3]. The major hypothesis towards the rising incidence of *Clostridium difficile* infection are increased use of broad spectrum antibiotics [4] and airborne dissemination of spores [5]. *Clostridium difficile* are ubiquitous in the environment. A study by Al Saif and Brazier [5] on distribution of *Clostridium difficile* showed that the organism can be isolated from water, residences, raw vegetables and fecal sample of assorted farm animals [5]. However, its role in infection and pathogenesis in animals are poorly understood,

hence underestimated [6]. Similarly, another study by Emma *et al.*, [7] suggests the potential airborne dispersal of *Clostridium difficile* in hospital environment with physical contact as the mode of transmission. The gastrointestinal tract of colonized or infected patients is the most frequent reservoir while transient carriage of *Clostridium difficile* on the hands of healthcare workers may lead to transmission [7]. *Clostridium difficile* disease has been traditionally regarded as a nosocomial infection in human especially in patient receiving prolonged antibiotic therapy [8]. Despite the recognition of *Clostridium difficile* infection and its well-known link with antibiotic use, incidence of infection may still increase [9]. Investigating the presence of *Clostridium difficile* in the hospital ward would bring about awareness on the need for better treatment, prevention and management of patients in the hospital.

## Materials and Method

### Sample Collection

Specialist Hospital Yola (SHY) and Federal Medical Centre Yola (FMCY) are the secondary and tertiary health facilities respectively within the State, hence the study area. One hundred and fifty (150) samples were collected, 75 from each hospital using sterile swab moistened with normal saline. Samples were collected within the medical wards, intensive care unit (ICU) and female surgical ward. The sites sampled included patient's bed sheets, bed railings, table tops, toilets and windows.

### Isolation of *Clostridium difficile*

Cycloserine Cefoxitin fructose broth (CCFB) and Cycloserine Cefoxitin fructose Agar (CCFA) composed of 4% proteose peptone, 0.01% MgSO<sub>4</sub>, 0.2% NaCl, 0.5% Na<sub>2</sub>HPO<sub>4</sub>, 0.1% KH<sub>2</sub>PO<sub>4</sub>, 0.6% fructose, 1.5% agar at pH 7.4 with added supplement containing 250 mg/L, D-Cycloserine, 8 mg/mL Cefoxitin and 5% sodium taurocholate were used for enrichment and isolation respectively. The swab containing the specimen was immersed in CCFB and incubated anaerobically at 37°C for 7 days. A loop full of cultured broth was then inoculated onto CCFA and incubated anaerobically at 37°C for 48 h. The isolates were identified by morphological characteristics, odor, biochemical test (oxidase, catalase and motility test) and Gram stain. The pure isolates were stored in CCFB and brain heart infusion broth (BHI) at -20°C prior to further studies.

### Antimicrobial Susceptibility Testing (MIC)

The inoculum of each isolate was prepared from overnight culture plate of the organism. A colony of each test organism was inoculated in 4 ml of sterile nutrient broth which was then incubated anaerobically for 2 h at 37°C. After the incubation, the turbidity of the suspension was matched to 0.5 McFarland Standard [10]. The susceptibility tests using the well diffusion method was carried out by determining the minimum inhibitory concentration (MIC) following the Clinical and Laboratory Standard Institute (CLSI, 2004) guidelines [11]. The antibiotic used includes metronidazole, ciprofloxacin, erythromycin, tetracycline and clindamycin. Varying concentrations were prepared by serial dilution from stock solution

of each antibiotic, which was prepared by dissolving each tablet of the antibiotics in 100 ml of sterile distilled water. 1 ml of standard inoculum of each isolate was then inoculated onto Mueller Hinton agar. A sterile swab was used to streak the surface of the agar [12]. Five (5) wells were made on each of the plates using a sterile cork borer which corresponds to the five antibiotics to be used. 20 µl of the various antibiotics concentration was dispensed into each of the well and was allowed to diffuse for 3 h on the bench. Plates were incubated anaerobically at 37°C for 48 h in an anaerobic jar. The MIC was recorded for each isolate.

## Results

A total of 18 (12%) isolates of *Clostridium difficile* were identified out of the 150 samples collected. Morphologically, the colonies are approximately 4 mm in diameter, ground glass appearance which has distinctive horse manure odor. Gram staining showed that organism is gram positive rods. Biochemical test further showed that isolates were both catalase and oxidase negative but were motile. *Clostridium difficile* was recovered in the various sites examined. In Specialist Hospital Yola, 8 (10.7%) positive *Clostridium difficile* isolates were obtained from the 75 samples examined. Two (2) were isolated from bed sheets in the ICU, and 3 each from male and female medical wards respectively (Table 1). No isolate was obtained from table top samples in all the wards within Specialist Hospital. Similarly, in the Federal Medical Centre Yola, 10 (13.3%) *Clostridium difficile* were isolated from the 75 samples in the various sites. Four (4) *Clostridium difficile* were isolated from the Female Surgical ward and 3 each from the male and female medical ward respectively while there was no isolate from bed railings in all the wards examined (Table 2). Antibiotic susceptibility tests revealed that *Clostridium difficile* isolated from the various hospital wards were resistant to all the antibiotics tested which included ciprofloxacin (MIC >64 µg/ml), erythromycin (MIC >64 µg/ml), metronidazole (MIC 64 µg/ml), tetracycline (MIC > 128 µg/ml) and clindamycin (MIC > 64 µg/ml) (Table 3).

## Discussion

The increase in *Clostridium difficile* infection (CDI) cases has spurred increasing concern regarding the growing segment of community associated infection distinct from infection acquired in healthcare settings [13]. Importantly, some evidence has shown that *Clostridium difficile* may be brought into the healthcare environment by asymptomatic carriers [14]. The reported carriage rates of *Clostridium difficile* in healthy adults have varied from 1% to 3% in Europe to up to 15% in Japan [15].

This current study depicts that *Clostridium difficile* were present on patient's beds, toilets, bed sheets and windows in the medical wards of the two hospitals. In Specialist Hospital, 2 isolates obtained from the ICU were from the drip stand and bed sheets. Four (4) out of the 25 samples examined in the Female surgical ward of the Federal Medical Center Yola were positive for *Clostridium difficile* out of which 2 were from the toilet samples. Several studies have reported the wide spread presence

**Table 1:** Occurrence of *Clostridium difficile* in Specialist Hospital Yola (SHY) wards.

Site of Sampling	ICU		MMW		FMW		Total
	No of samples	No of Isolate (%)	No of samples	No of Isolate (%)	No of samples	No of Isolate (%)	
Toilets	5	-	5	-	5	2.67%	2 (2.67%)
Bed Railings	5	-	5	1 (1.33%)	5	-	1 (1.33%)
Bed sheets	5	2 (2.67%)	5	-	5	1 (1.33%)	3 (4%)
Table Top	5	-	5	-	5	-	-
Window	5	-	5	2 (2.67%)	5	-	2 (2.67%)
TOTAL	25	2 (2.67%)	25	3 (4%)	25	3 (4%)	8 (10.67%)

ICU = Intensive Care Unit, MMW= Male Medical Ward, FMW = Female Medical Ward

**Table 2:** Occurrence of *Clostridium difficile* in Federal Medical Center Yola (FMCY) wards.

Site of Sampling	FSW		MMW		FMW		Total
	No of samples	No of Isolate (%)	No of samples	No of Isolate (%)	No of samples	No of Isolate (%)	
Toilets	5	2 (2.67%)	5	1 (1.33%)	5	2 (2.67%)	(6.67%)
Bed Railings	5	-	5	-	5	-	-
Bed sheets	5	-	5	1 (1.33%)	5	-	1 (1.33%)
Table Top	5	1 (1.33%)	5	1 (1.33%)	5	-	2 (2.67%)
Window	5	1 (1.33%)	5	-	5	1 (1.33%)	2 (2.67%)
TOTAL	25	4 (5.33%)	25	3 (4%)	25	3 (4%)	10 (13.33%)

FSW = Female Surgical Ward, MMW= Male Medical Ward, FMW = Female Medical Ward.

**Table 3:** Minimum Inhibitory Concentration (MIC) of antibiotics against *Clostridium difficile* isolates.

Antibiotics	MIC Range (µg/ml)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	%
Ciprofloxacin	0.5-32	128	128	64	256	128	128	256	256	128	64	64	64	128	64	64	64	256	128	100
Clindamycin	0.5-32	64	64	128	256	128	128	256	256	64	64	64	64	128	128	64	128	128	256	100
Erythromycin	0.5-32	64	64	128	128	256	64	128	128	128	64	64	64	64	128	128	256	128	128	100
Metronidazole	0.5-32	128	128	256	64	256	64	128	128	128	64	64	128	64	128	64	128	128	128	100
Tetracycline	1-62	128	128	128	128	256	256	128	128	256	256	128	128	128	128	256	256	128	128	100

of *Clostridium difficile* in hospital wards and on the hands of nursing personnel [16]. Environmental sample contamination with *Clostridium difficile* spores has been demonstrated in 34-58% of sites in hospital wards [17]. Adegoyega also reported that 7 out of the 60 samples examined in the medical wards were *Clostridium difficile* positive. A recovery rate of 12.5% recorded from the LUTH hospital (Lagos University Teaching Hospital) environment suggest that the main route of transmission may be through hospitalized patient [18].

In another studies carried out by Akhi *et al.* results showed that out of 70 *Clostridium difficile* isolates which were cultured as of first time in North West Iran, 18% (18 out of 100), 10.37% (14 out of 135), 32% (16 out of 50) and 44% (22 out of 50) were isolated from staff, hospital environment, patients at first day of admission and the same patients after seven days of hospitalization respectively. Six (6) patients (12%) were reported to be colonized by *Clostridium difficile* during days of hospitalization [19]. The presence of *Clostridium difficile* spore in the surrounding environment can be a source of transmission to individuals. An infected patient excretes more than 10<sup>5</sup> *Clostridium difficile* per gram of feces, and spores may survive on contaminated surfaces for months [20].

Nosocomial transmission of *Clostridium difficile* is believed

to occur primarily via the contaminated hands of healthcare workers or via environmental (including healthcare equipment) contamination and less commonly by direct patient-to-patient spread [15]. Contamination of healthcare workers' hands can lead to and result from contamination of the environment. Thus, the prevalence of healthcare worker contamination with *Clostridium difficile* correlates with the level of environmental contamination [21]. However, proof that reducing environmental *Clostridium difficile* can decrease the incidence of infection is lacking. Some other studies have reported that the environment surrounding both symptomatic and asymptomatic patient serve as a reservoir of *Clostridium difficile* [22].

Furthermore, Best *et al.* [20] reported that though the spores of *Clostridium difficile* can occasionally spread through the air but can be easily transmitted through the hands of the hospital staff [20]. In another study carried out in Kuwait by Rotimi *et al.* [23] reported that the acquisition rate of *Clostridium difficile* increased from 5.9 % to 36 % during a 4 to 53 days of hospitalization in various ward [23]. In another study by Conly [24], the risk of colonization by *Clostridium difficile* was found to increase in 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

[24]. Antimicrobial susceptibility test (MIC) showed resistance to the antibiotic tested which was contrary to the idea by most clinical laboratories which assume that all *Clostridium difficile* strains are susceptible to metronidazole and vancomycin [25]. Similarly, Wong *et al.* [26] reported about a clinical isolate that is highly resistant to metronidazole and vancomycin [26]. Another studies by Palaez *et al.* [27] also reported a 6.3% rate resistance to metronidazole amongst isolates of *Clostridium difficile*. The organism is reported to have developed resistance to some antibiotics, most notably the third generation cephalosporin's, clindamycin, and recently the fluoroquinolones class [27].

Reports showed that fluoroquinolones were responsible for 55% of infection during the hyper virulent *Clostridium difficile* outbreak in Quebec in the mid 2000's [27]. As these antibiotics are widely used, the most prevalent epidemic *Clostridium difficile* strains are often resistant to these common antibiotics. Vancomycin and metronidazole are effective antibiotics currently in use to treat *Clostridium difficile* infection; however, treatment with metronidazole has required an increase in the dosage for effective treatment of symptoms [28]. The optimum method for decontaminating hospital environments contaminated with *Clostridium difficile* remains controversial. Hypochlorite was found to be more effective than a quaternary ammonium solution in a bone marrow transplant (BMT) unit, but not in an ITU or a general medical ward [29]. The same workers reported a reduction in *Clostridium difficile* diarrhea rates in the BMT unit after use of the hypochlorite solution, but no reduction on the other wards studied. When the use of ammonium solution was restarted, rates of *Clostridium difficile* diarrhea rose, suggesting that hypochlorite solution is effective at reducing the risk of infection in high-risk clinical areas [30].

## Conclusion

The study confirmed the presence of drug resistant *Clostridium difficile* in the hospital environment. Contamination of healthcare workers' hands can lead to increased infections and re-contamination of the environment. Thus, the rate of contamination with *Clostridium difficile* correlates with the level of healthcare worker's contamination. Symptomatic patients should be isolated or cohort nursed, and hand hygiene is a key intervention to prevent nosocomial spread.

## References

1. Barbut F, Gariazzo B, Bonne L, et al. Clinical features of *Clostridium difficile*-associated infections and molecular characterization of strains: result of a retrospective study, 2000-2004. *Infection Control Hospital Epidemiology*. 2007; 28: 31-139.
2. Cookson B. Hyper virulent strains of *Clostridium difficile*. *Postgraduate Medical Journal*. 2007; 83: 291-295.
3. Dallal RM, Harbercht BG, Boujoukas AJ, et al. Fulminant *Clostridium difficile*: an underappreciated and increasing cause of death and complications. *Annual Surgery*. 2002; 235:

- 363-372.
4. Kyne L, Hammel MB, Polavaram A, et al. Health care costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. *Clinical Infectious Diseases*. 2002; 14: 346-335.
5. Al Saif N, Brazier JS. The distributions of *Clostridium difficile* in the environment of south Wales. *Journal of Medical Microbiology*. 1996; 45: 133-137.
6. Rupnik M. Is *Clostridium difficile* -associated infection a potentially zoonotic and food borne disease? *Clinical Microbiology Infections*. 2007; 13: 457-459.
7. Emma LS, Warren N, Fawley WN, et al. The potential for Airborne Dispersal of *Clostridium difficile* from Symptomatic patients. *Clinical infectious Diseases*. 2010; 50: 1450-1455.
8. Leffler DA, Lamont JT. *Clostridium difficile* infection. *New England Journal of Medicine*. 2015; 372: 1539-1540.
9. Stone S, Kibbler C, How A, et al. Feedback is necessary in strategies to reduce hospital acquired infection. *British Medical Journal*. 2000; 321: 302-303.
10. Drummond LJ, McCoubrey J, Smith DG, et al. Changes in sensitivity patterns to selected antibiotics in *Clostridium difficile* in geriatric in-patients over 18-month period. *Journal of Medical Microbiology*. 2003; 52: 259-263.
11. Clinical and Laboratory Standards Institute. (2004). Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard, 6th ed. NCCL document M11-A6. Wayne, PA: NCCLS. [https://clsi.org/media/2577/m11-ed9\\_sample.pdf](https://clsi.org/media/2577/m11-ed9_sample.pdf)
12. Mutlu E, Wroe AJ, Sanchez-Hurtado K, et al. Molecular characterization and antimicrobial susceptibility patterns of *Clostridium difficile* strains isolated from hospitals in South-east Scotland. *Journal of Medical Microbiology*. 2007; 56: 921-929.
13. Lucet JC, Rigaud MP, Mentre F. Hand contamination before and after different hand hygiene techniques; a randomized clinical trials. *Journal of Hospital Infection*. 2000; 50: 276-280.
14. Limbago B, Long C, Thompson A, et al. *Clostridium difficile* strains from community associated infection. *Journal of Clinical Microbiology*. 2009; 47: 3004-3300.
15. Mulligan ME. *Clostridium difficile* -its role in intestinal disease, London. Academic press. 2008; 229-256.
16. Wilcox MH. Gastrointestinal disorders and pseudomembranous colitis. *Best Practice Research Clinical Gastroenterology*. 2003; 17: 475-493.
17. Mcfarland LY, Mulligan ME, Kwok RY, et al. Nosocomial acquisition of *Clostridium difficile* infection. *New England Journal of Medicine*. 1989; 320: 204-210.
18. Adegboyega TT. Environmental sources *Clostridium difficile* in Lagos State, Nigeria. *Journal of Health, Medicine and Nursing*. 2014; 3: 1-5.
19. Akhi MT, Tahere P, Behruv N, et al. Antimicrobial

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- susceptibility of *Clostridium difficile* isolated from different sources of Imam Reza Hospital, Tabriz. African Journal of Microbiology Research. 2011; 5: 2946-2949.
20. Best ER, Fawley WN, Pamell P, et al. The potential for airborne dispersal of *Clostridium difficile* from symptomatic patients. Clinical Infectious Diseases. 2010; 50: 1450-1457.
  21. Fawley WN, Wilcox MH. Molecular typing of endemic *Clostridium difficile* infection. Epidemiology of Infection. 2001; 126: 343-350.
  22. Verity P, Wilcox MH, Fawley W, et al. Prospective evaluation of environmental contamination by *Clostridium difficile* in isolation side rooms. Journal of Hospital Infection. 2001; 49: 204-209.
  23. Rotimi VO, Mokadda EM, Jamak WY, et al. Hospital acquired *Clostridium difficile* infection amongst ICU and burn patient in Kuwait. Principles of Practical Medicine. 2002; 11: 23-28.
  24. Conly JM. Clostridium difficile-associated diarrhea. The new source of the health care facility. Canadian Journal of Infectious Disease. 2002; 11: 25-27.
  25. Baines SD, O'Connor R, Freeman J, et al. Emergence of reduced susceptibility to Metronidazole in *Clostridium difficile*. Journal of Antimicrobial Chemotherapy. 2008; 62: 1046-1052.
  26. Wong SS, Woo PC, Luk WK, et al. Susceptibility testing of *Clostridium difficile* against Metronidazole and Vancomycin by disc diffusion and E-test. Diagnostic Microbiology Infectious Disease. 1999; 34: 1-6.
  27. Palaez T, Alcalá L, Alonso R, et al. Reassessment of *Clostridium difficile* susceptibility to Metronidazole and Vancomycin. Antimicrobial Agents Chemotherapy. 2002; 46: 1647-1665.
  28. Pepin J, Saheb N, Coulombe MA, et al. Emergence of fluoroquinolones as the predominant risk factor for *Clostridium difficile* associated diarrhea: a cohort study during an epidemic in Quebec. Clinical Infectious Disease. 2005; 41: 1254-1260.
  29. Johnson S, Gerding DN, Olson MM. Prospective, controlled study of vinylglove use to interrupt *Clostridium difficile* nosocomial transmission. American Journal of Medicine. 1990; 88: 137-140.
  30. Mayfield JL, Leet T, Miller J, et al. Environmental control to reduce transmission of *Clostridium difficile*. Clinical Infectious Diseases. 2000; 31: 995-1000.