# Prevalence of Diarrhea due to *Clostridium difficile* A-B toxins in a University Hospital in Northern Cyprus

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**Abstract**

The high increase in diarrhea cases draws the attention of microbiologists to take the necessary precautions against the epidemic that occurs in some regions. It has been found each year that the mortality and morbidity rate of this infection explains about 500,000 cases in the United States. This retrospective study focuses on the investigation of the rate of C. difficile in a university hospital. *Clostridium difficile* toxin A / B results of patients admitted to the North Cyprus Near East University hospital between 2015-2018 were retrospectively extracted from the hospital registry system. A total of 230 patient data were used in the study. Data variables used included demographic information, department, inpatient or outpatient treatment. No significant difference was found in the age category in terms of *Clostridium difficile* toxin A / B positivity (p = 0.822). The highest positive *C. difficile* toxin A / B ratio was found in 18.2% in the 20-44 age group, while it was 15.5% in the age group 45 and over. However, there was no statistically significant difference in the age group as the chi-square result gave p-value = 0.721. The distribution of this infection showed statistical significance between inpatients and outpatients with a p-value of 0.018. While 9.70% of positivity was detected in inpatients, it was 21.30% in outpatients. The high rate of *C.difficile* infection among outpatients is due to the unregulated guidelines in the use of antibiotics obtained from pharmaceutical stores. This study shows the inadequacy of rational use of antibiotics in practice, although the sale of antibiotics without prescription is prohibited in Northern Cyprus.

## 1.    Introductıon

*Clostridium difficile* is a nosocomial pathogenic bacteria that releases proinflammatory cytotoxins namely Toxin A and B that cause the common known *C. difficile* infection (CDI). These toxins of C. difficile cause damage and inflammation in the colon mucosa.A clinical picture ranging from self-limiting mild diarrhea to severe pseudomembranous enterocolitis develops.Toxin A induces the production of neurokinins and cytokines which serve a pivotal role in the pathogenesis of *C. difficile* infections. Toxin B is usually targeted by the popularly approved FDA drug "Bezlotoxumab" which comprises IGHV5-51 and IGKV3-20 [1]. CDI is mostly considered a healthcare-associated nosocomial infection, but some studies outside of healthcare facilities are also noted in countries where outpatient antibiotic use is common. From studies, prevalence of toxigenic *C. difficile* varies among the Asian populations. In 2015, Cheng *et al*. [2] reported the prevalence of *C. difficile* to be  19.2% in China using PCR-based technique on stool culture, while Thailand has 9.2% [3]. In the UK, patients aged 65 and above are diagnosed for the presence of *C. difficile* associated diarrhea (CDAD) without suspecting any risk factor so as to lower its prevalence [4, 5]. Based on the current guideline, metronidazole hydrochloride has been recommended as a first line of defense for the treatment of severe CDI cases, but vancomycin was recently reported to be more effective than metronidazole [6].

Persistence rise of CDI is observed globally in developing countries due to lack of proper and early diagnostic measures. There is a scarce studies   for the prevalence of *C. difficile* in Turkey and TRNC. Therefore, this study intends to investigate the prevalence of this infection in NEU hospital to start at a narrow view.

## 2.   Materıals and Methods

This retrospective study was conducted at Near East University Hospital, Turkish Republic of Northern Cyprus, TRNC.  The study covers the period of 3 years (1ˢᵗ 2015-2018) with a total number of 230 samples obtained from the record unit of Near East University Hospital. Data was mainly for *C. difficile* related diarrhea . Both in-patients and out-patients were included in the study. In the experimental process, **MiniVIDAS** (Biomérieux)  was used to test toxin A/B and as described by the manufacturer.

### 2.1. Statistical analysis

Statistical Package for the Social Sciences (SPSS) software version 20 was used to analyse variables. Continuous data such as gender and age, were analysed as percentage of total sample collected. Categorical data such as department were analysed using Chi-square test.

## 3.   Results and Discussion

A total of 230 respondents from demographic and clinical tests of patient data were used for this research and it was found that male accounted for 47.00% and female with 53.00%. Patient ages result for those with less than 20 years accounted for 6.50%, between 20 to 44 years showed 43.00%, 45 to 64 years recorded 25.20% and greater than or equal to 65 years recorded also 25.20% respectively. The outcomes for the patient test result showed that those with positive were 16.10% and those with negative were 83.90% and lastly the category of admitting patients in the hospital revealed that in-patient were the least with 44.80% and out-patient have the highest with 55.20% (Table 1).

**Table 1**. Demographic and Clinical test characteristics of the patients (n = 230).

| Variables | N (%) |
|---|---|
| Gender | |
| Male | 108(47.00%) |
| Female | 122(53.00%) |
| Age | |
| <20 years | 15(6.50%) |
| 20-44 years | 99(43.10%) |
| 45-64 yeears | 58(25.20%) |
| ≥65 years | 58(25.20%) |
| Test outcome | |
| Positive | 37(16.10%) |
| Negative | 193(83.90%) |
| Patient admittance category | |
| In-Patient | 103(44.80%) |
| Out-Patient | 127(55.20%) |

Distribution of toxin-positive and toxin-negative strains in different hospital units shows internal medicine with the highest number of *C. difficile* A-B toxins, of which 27(24.30%) tested positive while 84(75.70%) tested negative . Gastroenterology unit with 30 patients is the second most dorminant. But the results of gastroenterology, general surgery, oncology and orthopedics and traumatology units do not record any positive test while the only two patients found in the brain surgery unit tested all positive with no negative test result. While in total, 37(16.10%) of all the 230 patients considered in the study tested positive and 193(83.90%) tested negative (Table 2).

**Table 2**. Distribution of toxin-positive and toxin-negative strains in different hospital units

| Units | No. of C. difficile A-B toxin (%) | No. of positive A-B toxin CD (%) | No. of negative A-B toxin CD (%) |
|---|---|---|---|
| Brain Surgery | 2(0.90%) | 2(100.00%) | 0(0.00%) |
| Cardiology | 7(3.00%) | 2(28.60%) | 5(71.40%) |
| Gastroenterology | 30(13.00%) | 0(0.00%) | 30(100,00%) |
| General Surgey | 2(0.90%) | 0(0.00%) | 2(100,00%) |
| Infectious Diseases | 26(11.30%) | 2(7.70%) | 24(92.30%) |
| Internal Medicine | 111(48.30%) | 27(24.30%) | 84(75.70%) |
| Intensive Care Unit | 8(3.50%) | 1(12.50%) | 7(87.50%) |

| | | | |
|---|---|---|---|
| Laboratory | 18(7.80%) | 2(11.10%) | 16(88.90%) |
| Pediatric | 12(5.20%) | 1(8,30%) | 11(91.70%) |
| Oncology | 12(5.20%) | 0(0.00%) | 12(100.00%) |
| Orthopedic and Traumatology | 2(0.90%) | 0(0.00%) | 2(100.00%) |
| Total | 230 (100.00%) | 37(16.10%) | 193(83.90%) |

On gender basis, reveal 18(16.70%) of the male patients were positive and 90 (83.30%) tested negative.While in the female 19 (15.60%) tested positive and 103 (84.40%) tested negative. The chi-square statistic shows that the Gender categories are not statistically significantly different in terms of test outcome ($\chi^2$= 0.051, p= 0.822). It can be inferred that gender does not have any significance association with the test result outcome.

Furthermore, higher percentage was seen from the male patients compared to female patients. Subsequently from both the in-patient and out-patient result for *Clostridium difficile* A-B toxin showed that those tested negatives were higher than those tested positive. And the result from cross tabulation showed no statistically significant difference between genders versus test for *C. difficile* A-B toxin, and between age categories versus test for clostridium difficile A-B Toxin, but contrary patient admittance status was statistically significant.

On age grop, the *Clostridium difficile* A-B toxin, positive *Clostridium difficile* A-B toxin and negative *Clostridium* A-B toxin showed that less than 20 years were 100.0%, 6.70% and 93.30% respectively. Age 20 to 44 showed 100.0%, 18.20% and 81.80%. Age 45 to 64 gave 100.0%, 15.50% 84.50% respectively and age greater or equal to 65 gave 100.)%, 15.50% and 84.50% respectively.Furhermore, for the ages of positive A-B toxin and negative A-B toxin showed that less than 20 years is represented with 6.70% and 93.30%. 20 to 44 years is represented with 18.20% and 81.80%. 45 to 64 years is represented with 15.50% and 84.50%. And lastly greater than or equal to 65 years represent with 15.50% and 84.50% respectively.

On hospitalization status, the result of admittance category of in-patient showed that, those tested positive *Clostridium difficile* A-B toxin were 9.70% and negative were 90.30% while for those out- put patient that tested positive clostridium difficile A-B toxin gave 21.30% and for negative showed 78.70%. The Chi-square test result showed that patient admittance status was statistically significant. This means the condition of being admitted as an In-patient or as an Out-Patient have significance association with the test result outcome. The reported rise (20-28%) of CDI cases in Europe and North America are community associated infections [7]. Several interventions are needed to put in place in the case of CDAD outbreak; among few is the isolation of affected patients to a particular section of the hospital or clinic, proper hygiene of wards and change/regulation of the given antibiotic. Among the major concern as a result of rise in the prevalence of this infection is the persistent rise in the use and misuse of many antibiotics. Previously, James et al., [8] investigated the incidence usage of antibiotics and infections that are related to health care in Northern Cyprus. The study found a statistical correlation between gender and duration of hospitalization with prevalence of health associated infections; with about 60% of inappropriate use of antibiotics. It is now necessary to investigate the prevalence of *C. difficile* toxin A-B in some parts of Northern Cyprus. This study is aimed at investigating the prevalence of *C. difficile* in Near East University Hospital.

Following the result of analysis, from table 1, it revealed that female patients' response was higher compared to male patients, while their ages category showed that 20 to 44 age groups recorded a higher percentage and less than 20 years accounted for the least percentage. 45 years and above seems to maintain

constant prevalence of the infection. This study shows prevalence at lower age when compare to previous studies where high rate start at age greater than 65 [9]. Other previous studies also reported increase in severe *C. difficile* rate in children with bloody diarrhea [10, 11], while a study a recent study by Liao et al. [12] reported a high prevalence of 86.36% in hospitalized adults. Another recent study by Curcio et al [13] reported 15% prevalence from different regions which include developing Asia, Africa-Middle East, China and Latin America. The study is a systematic literature search from various search engines and database; and comprises both community and hospital related cases. However, in this study, patients with negative results were higher compared to those tested positive. Patients out of admission (out-patients) were higher to those patients that are on admission (in-patients). This may be due to strong and well standardized antibiotic policy adopted by the Near East University Hospital. On the other hand, Xiao et al. [14] suggest that increase in publicity awareness among both patients and clinicians should be given necessary attention so as to curb the spread of the infection.

Different units of the hospital show varying percentages of the infection with internal medicine unit recording the highest number of *Clostridium difficile* A-B toxins, but also show 75.70% of the recorded patients to be negative. Surprisingly, gastroenterology unit in our study recorded no positive result (100% negative). This is contrary to the study of Zhou et al [9] were gastroenterology department reported a prevalence of 70.4% among patients. Our study reported a prevalence of 16.10% C. difficile in our university hospital. This is higher than other reported studies [15].The differences in incidence rate of *C. difficile* maybe due to technological advances and diagnostic expertise in different regions [5, 16] and also exposure to many levels of the known risk factors.

This study presents the prevalence of *C. difficile* in NEU Hospital and shows a rise in the rate from outpatients, which is due to the poor regulation in the use of antibiotics among outpatients. Other reasons may be transmission of the infection in the environment via contact and the diet consumed.  This suggests the reverse of regulation on the use of antibiotics in TRNC as earlier done on 1st April 2016. There is also a need to take representative data from all or different hospitals within North Cyprus so as to obtain a larger population, as the results  of this study are limited to that of the record unit of Near East University, TRNC.

**Acknowledgment**

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