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Original article

Clostridium difficile A-B Toxins as a Cause of Diarrheal Disease: Data from 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 that each year, the mortality and morbidity rates of this infection account for 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, and 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 a 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 a prescription is prohibited in Northern Cyprus.
Keywords. *Prevalence, Clostridium difficile, Northern Cyprus.*

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Introduction

C. difficile is a nosocomial pathogenic bacterium 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 (Orth et al., 2014).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, the prevalence of toxigenic *C. difficile* varies among the Asian populations. In 2015, Cheng *et al*. (2015) reported the prevalence of *C. difficile* to be 19.2% in China using a PCR-based technique on stool culture, while Thailand has 9.2% (Putsathit *et al*., 2017). In the UK, patients aged 65 and above are diagnosed with the presence of *C. difficile-associated* diarrhea (CDAD) without suspecting any risk factor, so as to lower its prevalence (Barbut *et al*., 2003; Planche *et al*., 2008). Most studies tend to look for molecules that will block the pathogenesis of this infection. Metronidazole and vancomycin are popularly known for the treatment of CDI, especially in severe conditions. 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 (Stevens et al., 2017). Occurrence of *C. Difficile* was also reported in animal sources, as reported by (Özgen and Yildirim, 2021). Another study from Turkey suggested the possible link between transmission and survival of C. Difficile through the food chain (Ersöz and Coşansu, 2018). It is clear that the occurrence of the organism is the main factor in the production of the toxins,

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Persistent rise of CDI is observed globally in developing countries due to a lack of proper and early diagnostic measures. There are 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 with a narrow view.

Materials 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. The data were mainly for *C. difficile-related* diarrhea. Both inpatients and outpatients were included in the study. In the experimental process, MiniVIDAS (Biomérieux) was used to test toxin A/B and as described by the manufacturer.

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 a percentage of the total sample collected. Categorical data, such as department, were analysed using the Chi-square test.

Result

230 respondents from demographic and clinical tests of patient data were used for this research, and it was found that males accounted for 47.00% and females for 53.00%. Patient ages resulted in those with less than 20 years accounting for 6.50%, between 20 to 44 years showing 43.00%, 45 to 64 years recording 25.20% and greater than or equal to 65 years recording 25.20% respectively. The outcomes for the patient test result showed that those with positive results were 16.10% and those with negative results were 83.90% Lastly, the category of admitting patients in the hospital revealed that inpatients were the least, with 44.80% and outpatients had 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 years	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. The gastroenterology unit with 30 patients is the second most dominant. But the results of gastroenterology, general surgery, oncology, 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. In total, 37(16.10%) of all the 230 patients considered in the study tested positive, and 193(83.90%) tested negative (Table 2).

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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 Surgery	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%)
Orthopedics and Traumatology	2(0.90%)	0(0.00%)	2(100.00%)
Total	**230 (100.00%)**	**37(16.10%)**	**193(83.90%)**

Analysis of the gender category (Table 3) reveals that 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 (χ^2= 0.051, p= 0.822). It can be inferred that gender does not have any significant association with the test result outcome.

Table 3. Chi-Square Test (Gender Versus Test for Clostridium difficile A-B toxin)

Gender	Positive strain n (%)	Negative strain n(%)	χ^2	*p*
Male	18(16.70)	90(83.30)	0.051	0.822
Female	19(15.60)	103(84.40)		

The results from (Table 4a) for 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% and 84.50% respectively, and age greater than or equal to 65 gave 100.)%, 15.50% and 84.50% respectively.

Results from table 4b 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%. The 20- 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 represents 15.50% and 84.50% respectively. However, the cross-tabulation outcomes revealed there was no statistically significant difference between age category and test for Clostridium difficile A-B Toxin.

Table 4A. distribution of toxin-positive and toxin-negative C. difficile in different age groups

Age group	No. of C. difficile A-B toxin (%)	No. of positive CD A-B toxin (%)	No. of negative A-B toxin (%)
<20 years	15(100.00)	1(6.70)	14(93.30)
20-44 years	99(100.00)	18(18.20)	81(81.80)
45-64 years	58(100.00)	9(15.50)	49(84.50)
≥65 years	58(100.00)	9(15.50)	49(84.50)

From Table 5, results for the status of admittance category of in-patients showed that those who tested positive for Clostridium difficile A-B toxin were 9.70% and negative were 90.30% while for out-patients who tested positive for Clostridium difficile A-B toxin were 21.30% and those who tested negative were 78.70%. The Chi-square test result showed that patient admittance status was statistically significant. This means the condition of being admitted as an Inpatient or as an outpatient has a significant association with the test result outcome.

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Table 4 B. Chi-Square Test (Age category Versus Test for Clostridium difficile A-B Toxin)

Age	Positive A-B toxin n (%)	Negative A-B toxin n (%)	χ^2	*p*
<20 years	1(6,70)	14(93.30)	1.336	0.721
20-44 years	18(18.20)	81(81.80)		
45-64 yeears	9(15.50)	49(84.50)		
≥65 years	9(15.50)	49(84.50)		

Table 5. Chi-Square Test (Patients Admittance Status Versus Test for Clostridium difficile A-B toxin)

Patient Status	Positive Test n(%)	Negative Test n (%)	χ^2	*p*
In-Patients	10(9.70)	93(90.30)	5.622	0.018
Out-Patients	27(21.30)	100(78.70)		

Discussion

The reported rise (20-28%) of CDI cases in Europe and North America is a community-associated infection (Kuijper *et al.*, 2006). Several interventions are needed to put in place in the case of CDAD outbreak; among them is the isolation of affected patients to a particular section of the hospital or clinic, proper hygiene of wards, and the change/regulation of the given antibiotic. Among the major concerns resulting from the rise in the prevalence of this infection is the persistent increase in the use and misuse of many antibiotics. Previously, Jame et al. (2018) investigated the incidence of antibiotic usage and healthcare-related infections 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), revealed that female patients' response was higher compared to male patients, while their age category showed that the 20 to 44 age group recorded a higher percentage, and those less than 20 years accounted for the least percentage. People 45 years and above seem to maintain a constant prevalence of the infection. This study shows prevalence at a lower age when compared to previous studies, where high rates start at an age greater than 65 (Zhou et al., 2019). Other previous studies also reported an increase in severe C. difficile rate in children with bloody diarrhea (Karaaslan et al., 2016; Schwartz et al., 2014), while a recent study by Liao et al. (2018) reported a high prevalence of 86.36% in hospitalized adults. Another recent study by Curcio et al (2019) reported a 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 databases, and comprises both community and hospital-related cases.

However, in this study, the rate of negative results was higher compared to those who tested positive. Patients out of admission (out-patients) were higher than those patients who are on admission (in-patients). This may be due to a strong and well-standardized antibiotic policy adopted by the Near East University Hospital. On the other hand, Xiao et al. (2020) suggest that an 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 the 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, the gastroenterology unit in our study recorded no positive results (100% negative). This is contrary to the study of Zhou et al (2019), where the gastroenterology department reported a prevalence of 70.4% among patients.

From (Table 3), a higher percentage was seen in the male patients compared to the female patients. Subsequently, both the in-patient and out-patient results for *Clostridium difficile* A-B toxin showed that the tested negatives were higher than the tested positives. 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 to patient admittance status was statistically significant.

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Our study reported a prevalence of 16.10% C. difficile in our university hospital. This is higher than other reported studies (Prattingerová et al., 2019). The differences in incidence rate of *C. difficile* may be due to technological advances and diagnostic expertise in different regions (Planche et al., 2013; Polage et al., 2015), and also exposure to many levels of the known risk factors.

Conclusion

This study presents the prevalence of *C. difficile* in NEU Hospital and shows a rise in the rate among outpatients, which is due to the poor regulation of 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 the TRNC, as was 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.

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