Retrospective analysis of risk factors, demographic incidences and clinical symptoms for Hospital-acquired *Clostridioides difficile* infection

by

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University of Pittsburgh, 2023

Abstract

Clostridioides difficile (C. difficile) is a significant public health concern due to the widely spreading hospital-acquired infection primarily leading to acute diarrhea. We collected information on hospital-acquired *C. difficile-positive* patients from a single academic hospital in Pittsburgh, PA. We studied the use of antibiotics, proton pump inhibitors, and feed tubes as risk factors associated with *C. difficile* infection (CDI) and performed the demographic analysis with respect to age, gender, and race for these patients. We also assessed the clinical symptoms – abdominal pain/cramping / tender distension, diarrhea / loose stool, fever > 38 ^{o}C, and High WBC – associated with these patients.

CDI patients who used antibiotics are in significantly greater proportion than non-users. The proportion of proton pump inhibitors and feed tube users were not different. The proportion of CDI patients older than 60 years and those of white race were significantly greater than patients less than 60, and non-whites respectively. CDI occurs equally among males and females. However, we did not observe any clear relationship between *C. difficile* toxin production and the above factors. As expected, the CDI patients exhibited the following clinical symptoms: (a) 57.4% exhibited abdominal pain, cramping or tender distension, (b) a significantly greater proportion reported diarrhea or loose stool and (c) had a high WBC count. Also, about 30% of the CDI patients reported high fever. Overall, from the retrospective analysis of CDI patient record from one

hospital, we conclude that certain medications could act as risk factors for CDI and also affect certain population disproportionately.

Public health significance: *Clostridioides difficile* infection is becoming a significant public health concern as the disease severity and the proportion of individuals infected in hospital settings steadily increase. It causes severe infectious diarrhea that can significantly impact people's lives physically and emotionally.

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Preface

I would like to thank my essay advisor and committee chair, Dr. Mohamed Yassin, who took time out of his busy schedule and provided me with unwavering support and guidance throughout the completion of my master's thesis. His insights and expertise helped to shape my research and improve the quality of my essay. I am deeply grateful for his dedication and patience, and I am fortunate to have had such an inspiring mentor.

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Nomenclature

C. difficile	*Clostridioides difficile*
CDC	Centers for Disease Control and Prevention
CDI	*Clostridioides difficile* infection
DHQP	Division of Healthcare Quality Promotion
ICU	Intensive care unit
FDA	Food and Drug Administration
NHSN	National Healthcare Safety Network
PEG	Polyethylene glycol
PPI	Proton pump inhibitor

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1.0 Introduction

Clostridium difficile (*C. difficile*) is a widespread hospital-acquired infection primarily leading to acute diarrhea among hospitalized people, which has emerged in recent years with more significant morbidity and mortality, more so in the developed world (Lessa et al., 2015). According to the Centers for Disease Control and Prevention (CDC), more than 500,000 Americans suffer from *C. difficile* infections (CDI) in a single year. 20% of these patients also exhibit recurrence (Lessa et al., 2015). Such large numbers have led to financial implications. According to an estimate, the burden of CDI in health care costs for hospitals is more than a vast sum of $4.8 billion each year (Lessa et al., 2015).

The Gram-positive, spore-forming, anaerobic bacillus *Clostridium difficile* is widely distributed in the intestinal tract and is now considered as one of the most common hospital-acquired infections (Smits et al., 2016). If infected, the patients exhibit a diverse range of symptoms - from asymptomatic carrier status, through various degrees of diarrhea, and a few times to the most severe and life-threatening colitis that results in the death of the patient. A preferred diagnosis of the infection is by direct detection of *C. difficile* toxins in the feces of patients. However, there is no preferred single stand-alone test to confirm CDI. In fact, the growing size of hospital-acquired infections around the country and the difficulties of establishing effective infection control are a matter of concern that justify various guidelines issued by Public Health Organizations such as the CDC. Many factors influence the chance of acquiring or are considered as risk factors for CDI. A proper identification of risk factors could be beneficial in many ways. As for example, if by chance it is possible to eliminate risk factors, this will certainly reduce the incidence of the infection. Another reason is that high-risk individuals might be monitored more

closely to enable the early detection of infection and timely institution of treatment and infection control precautions. Also, in the case of non-removable risk factors, the local variation of *C. difficile* can be compared, and infection rates in different hospitals could be made, which can help in the compilation and comparison of data nationwide. Known risk factors for CDI are previous hospitalization, underlying disease, advanced age, and, most importantly, the use of antibiotics. Advanced age contributes to the susceptibility to *Clostridioides difficile* and thus is *a* significant risk factor for the infection. It has been reported that *C. difficile* occurred more frequently in the >60-year age group (Spina et al., 2015).

1.1 *Clostridioides difficile*

Clostridioides difficile is a gram-positive, anaerobic, spore-forming, toxin-producing bacillus, sometimes referred to as *Clostridioides difficile* (Czepiel et al., 2019; Smits et al., 2016). It is a potentially life-threatening bacterium that can cause liquid diarrhea and may lead to more severe intestinal conditions such as the chronic digestive condition colitis (inflammation of the large intestine) (Czepiel et al., 2019). This organism can be found in water, air, the intestinal tract of humans and animals, animal feces, hospital surfaces, and soil. The organism can optimally grow at a temperature of about 37º Celsius. *C. difficile* bacteria are known to survive on fecal surfaces for a long time. It is a challenge to kill this spore-forming pathogen compared to other bacteria. Spores of *C. difficile* are usually transmitted by the fecal-oral route. People can thus be infected if they touch items or surfaces contaminated with feces or spores and then touch their mouth or mucous membranes. *Clostridioides difficile* in healthcare settings can transmit from person to person, by a

contaminated environment, or via patient care equipment. However, some people may be asymptomatic carriers of *C. difficile*.

1.1.1 Prevalence of the infection

According to the Centers for Disease Control and Prevention (CDC), CDI appears to increase rapidly in the United States. It has become among the most common and challenging nosocomial disorders affecting all hospital wards in the country. In a 10-year retrospective US patient discharge study, it was found that the incidence of CDI among hospitalized adults in the United States nearly doubled from 2001-2010 (Reveles et al., 2014). Moreover, the study showed little evidence of improved patient mortality or length of stay in hospitals. As mentioned above, CDC indicates that *C. difficile* infects approximately half a million Americans yearly. Among those infected with *C. difficile,* about 83,000 patients had at least one recurrence, and approximately 29,000 patients had fatal outcomes within a month of the initial diagnosis, where 15,000 of these deaths could be directly attributed to CDI (Centers for Disease Control and Prevention, 2022).

1.1.2 Prevention of the disease

To prevent patients from catching *C. difficile,* healthcare workers, including doctors, nurses, patients, and visitors, should wash their hands with soap and water. This practice also significantly reduces healthcare-associated infections, along with *C. difficile* Alcohol rubs alone are considered not sufficient to eliminate *C. difficile*. Other practices that can prevent the spread of the disease within hospitals are -- patients with CDI should ideally have a single room, and visitors may need to wear a gown and gloves. In the offices within these healthcare centers, doctors

should be informed about such recent hospitalization if a patient comes in complaining of severe diarrhea. These practices help doctors in finding the reasons for diarrhea. Additionally, people can protect themselves from getting CDI. However, good hand hygiene is the best single action that common people can take to prevent themselves and their family members from CDI.

1.1.3 Surveillance system in place

The **National Healthcare Safety Network** (NHSN) is a secure, internet-based surveillance system that integrates patient and healthcare personnel safety surveillance systems managed by the Centers for Disease Control and their prevention section named Division of Healthcare Quality Promotion (CDC, DHQP). To report to NHSN, a LabID event is declared a positive CDI case if the laboratory test detects any *C. difficile* toxin A and toxin B or finds any toxin-producing *C. difficile* organisms in the culture of a stool specimen. A hospital-acquired CDI is thus registered when the laboratory confirms a positive *C difficile* toxin assay at least 72 hours after acquiring liquid stool samples.

1.2 Risk factor for CDI

The chances for CDI are high when people have been exposed to the following factors: Serious underlying illness that led to hospitalization; Extended length of stay in healthcare settings; Gastrointestinal surgery/gastrointestinal procedure; prolonged antibiotic usage; or have conditions that weakened their immune system; have a history of CDI; or/and fall under the category of

advanced age (Smits et al., 2016). Most importantly, the use of antibiotics is a known and well-researched risk factor for CDI (Loo et al., 2011; Slimings & Riley, 2014).

1.2.1 Antibiotics that elevate CDI

Exposure to antibiotics significantly increases the subsequent risk of hospital-acquired CDI (Forster et al., 2017; Loo et al., 2011; Slimings & Riley, 2014). The clinically relevant increase in *C. difficile* risk persists after adjustment for differences in patient-level antibiotic use and other patients- and ward-level risk factors (K. Brown et al., 2015). It is thought that there are differential effects of age, medication use, host immunity, and pathogen variables on healthcare-associated CDI and healthcare-associated *C. difficile* colonization (Loo et al., 2011). Several hospital-acquired CDI models exist that consistently demonstrate a good predictive ability for early risk assessments (Dubberke et al., 2011; Tabak et al., 2015). Nevertheless, all antibiotic classes are associated with CDI, but the literature suggests that the more common antibiotics leading to CDI are clindamycin, cephalosporins, and fluoroquinolones (K. Brown et al., 2015; Dubberke et al., 2011; Hensgens et al., 2011; Loo et al., 2011; Slimings & Riley, 2014; Smits et al., 2016; Tabak et al., 2015). It is known that antibiotic-induced dysbiosis of the protective intestinal microbiota often leads to *C. difficile* outgrowth and the related toxin production (Theriot et al., n.d.; Theriot & Young, 2015). In addition to the antibiotic class, the number of administered antibiotics, dose, and duration of therapy have been thought to contribute to risk factors for CDI. In these cases, the disruption of the intestinal flora persists for >3 months after antibiotic therapy, causing patients to remain susceptible to CDI development long after ending the treatment. The cumulative damage to the intestinal microbiota is sufficient to enable *C. difficile* even with low-risk antibiotics, such as trimethoprim and piperacillin-tazobactam (Stevens et al., 2011).

One reason for the seriousness of the infection with *C. difficile* is that they mainly occur through spore transmission (Theriot et al., n.d.). Spores are known to be resistant to heat, acid, and antibiotics. Additionally, the main protective barrier against CDI is the normal intestinal microflora. But antibiotics can destroy both good and bad bacteria, thus upsetting the natural balance in the body. When the balance of gut microorganisms is disrupted, *C. difficile* starts to dominate and colonize the large intestine, creating the first step of this infection. Also, *Clostridioides difficile* produces two potent toxins that are referred to as toxins A and B. These are thought to be primarily responsible for the bacterium's virulence and are considered significant contributors to the pathogenesis of antibiotic-associated gastrointestinal disease. In short, understanding CDI and colonization can have implications for its prevention and subsequent therapy.

1.2.2 Proton pump inhibitor's relationship to CDI

Proton pump inhibitor (PPI) administration has recently garnered considerable attention for its potential role in promoting CDI (Kwok et al., 2012; Loo et al., 2011; E. G. McDonald et al., 2015; Wombwell et al., 2018). PPIs are globally used and are among the most prescribed medications to suppress gastric acid, an important host defense mechanism to prevent the germination of ingested *C. difficile* spores. On the other hand, Prolonged usage of PPIs disrupts the healthy human gut microbiome. It induces alterations to the gut microbiome that may, in turn, facilitate the emergence of CDI (Seto et al., 2014). Despite the safety announcement by the US Food and Drug Administration (FDA) suggesting an adverse association of PPIs with CDI (US Food and Drug Administration, 2012), such that they increased the risk of this hospital-acquired infection, gastric acid suppressor usage remains ubiquitous.

According to the current status of medical literature, the nosocomial administration of PPIs has a positive role in the development of hospital-acquired CDI. In 5-year surveillance at a large urban medical center, an increase in PPI use and an increase in hospital-acquired CDI incidence were found to be correlated (Jayatilaka et al., 2007). The study found out that: (a) As overall PPI use increases, so do hospital-acquired CDI cases; (b) continuation of an outpatient PPI during hospitalization is associated with hospital-acquired CDI, and (c) newly initiated PPI increased risk of developing hospital-acquired CDI during hospitalization. Researchers also identified that the duration threshold at which PPI administration increases CDI risk could be as short as 1-2 days (Barletta et al., 2013). A duration of use threshold at which the risk of hospital-acquired CDI increased occurred with as little as one day of PPI in patients with a previous hospitalization and two days of PPI in previously un-hospitalized patients (Barletta et al., 2013). Patients admitted to the intensive care unit (ICU) also have a high risk for stress ulcer development, where PPI therapy was the most common treatment method. It was found that the development of hospital-acquired CDI was associated with a more extended ICU stay and increased ICU death (Buendgens et al., 2014). Thus, the above studies provide supportive evidence that PPI therapy in the hospital, even for short courses of 1-2 days, heightens the risk for hospital-acquired CDI. It is possible that reevaluating PPI use in the hospital and discontinuing chronic PPI therapy might minimize unfavorable effects leading to hospital-acquired CDI.

1.2.3 Tube feeding's role in CDI

A common but less recognized factor for CDI is the prolonged use of 'elemental diets,' a liquid meal replacement diet that has the required nutritional profile broken down into its most "elemental" form (Alvarez-Lerma et al., 2014; Larrainzar-Coghen et al., 2016; O'Keefe, 2010). It

is medically referred to as ‘enteral feeding,’ where the nutrition is taken through the mouth or through a tube that goes directly to the stomach or small intestine. The most common term for ‘elemental diets’ or ‘enteric feeding’ is ‘tube feeding.’ Usually, tube feeding involves a liquid food mixture containing protein, carbohydrates (sugar), fats, vitamins, and minerals, given through a tube into the stomach or small intestine. Such diets are totally absorbed within the small intestine and therefore deprive the colonic microbiota of their source of nutrition, namely dietary fiber, fructose oligosaccharides, and resistant starch. The resultant suppression of colonic fermentation leads to suppression of the “good” bacteria, such as butyrate-producers (butyrate being essential for colonic mucosal health) and bifidobacterial and the creation of a “permissive” environment for *C. difficile* colonization and subsequent infection (O’Keefe, 2010). To reduce the incidence of CDI, it was suggested that the author restrict non-residue tube feeds to critically ill patients with ileus and borderline gut function and possibly change to fiber or “prebiotic” containing formula only after the initial use (O’Keefe, 2009).

1.2.4 Laxatives and mix-ups with CDI

Non-antibiotic medications such as the osmotic laxative polyethylene glycol 3350 (PEG 3350) are also known to alter the microbiota. It is, however, unclear whether PEG helps clear *C. difficile* or impacts *C. difficile* susceptibility. There are similarities between the bacterial colonies of people with diarrhea and those with CDI. For this reason, diarrheal samples from patients taking laxatives are typically rejected for *C. difficile* testing. However, reports suggest no difference in underlying patient characteristics, the clinical presentation of CDI, CDI attributable outcomes, or CDI severity in patients with clinically significant diarrhea who received laxatives preceding CDI diagnosis compared with patients who did not receive laxatives (White et al., 2020). Thus,

precluding recent laxative users from CDI testing can confound and delay the CDI diagnosis and possible timely treatment. Therefore, improving diagnostic stewardship around *C. difficile* testing, particularly in surgical and ICU patients, is a significant opportunity and priority for the quality improvement (Carter & Malani, 2019).

Host factors such as advanced age (Bartlett, 1992; Keller & Surawicz, 2014), immunosuppression, prior hospitalization, and severity of underlying illness are also essential factors that contribute to the increased risk of CDI (Asempa & Nicolau, 2017). Aging alters important physiologic barriers to infection, ranging from changes in genitourinary physiology that impair bladder function to decreased gastrointestinal microbial diversity (Yoshikawa & Norman, 2017). Additionally, the complex changes in the immune system related to advancing age, collectively called immunosenescence, play a crucial role in increased susceptibility in the elderly population. Immunosenescence is often associated with a decrease in T-cell and B-cell counts and a decline in cell function (Zheng et al., 1997). This age-related pathophysiology thus enhances the risk for morbidity and mortality, where it limits the ability of old adults to respond to microbes. The above reason supports the idea that older adults exhibit increased CDI compared to younger ones.

Despite proactive infection control measures such as hand hygiene, antibiotic stewardship, environmental cleaning, and a strict surveillance system, *C. difficile*-associated disease remains problematic. About 35% of patients infected with *C. difficile* appear unrelated to antibiotic use or did not have prior exposure at healthcare facilities (Furuya-Kanamori et al., 2015). Also, nearly 1–3% of healthy adults and 15–20% of infants are asymptomatic *C. difficile* carriers with a normal microbial gut community (Furuya-Kanamori et al., 2015). It would be useful for clinicians to have data on the medical conditions associated with *C. difficile*. As in some reports, the severity of the infection suggested that the patients may exhibit a temperature above 38.3°C (Kelly & LaMont,

2008). The studies on clinical factors associated with *C. difficile* suggest that a substantial proportion of patients, especially those with WBC counts of > 11,000 cells/mm3, have the infection. A white blood cell (WBC) count increase may be associated with hospital-acquired CDI as a sign of an ongoing infection and inflammation in the body. This is because WBCs are a part of the body's immune response to a disease, and a higher WBC count can indicate an increased presence of infection-fighting cells in the body. Abdominal pain is also a common symptom associated with hospital-acquired CDI. The pain may result from inflammation and irritation caused by the toxins produced by the *C. difficile* bacteria in the gastrointestinal tract. The severity of abdominal pain can range from mild to severe and can be accompanied by other symptoms such as abdominal tenderness or cramping.

1.3 Public health significance of the study

CDI is becoming a significant public health concern as the disease severity and the proportion of individuals infected in hospital settings steadily increase. It is a severe infectious disease that can significantly impact people's lives, both physically and emotionally. *C. difficile* is a multi-resistant pathogen recognized as the leading cause of diarrhea in healthcare settings. It is considered one of the most important public health threats because it is associated with antibiotic treatments and high morbidity and mortality (Centers for Disease Control and Prevention, 2013). Considering the various risk factors related to the infection – such as the use of broad-spectrum antibiotics, underlying severe illness, conditions that weaken the immune system, and higher susceptibility of elderly people – raises serious public health safety concerns about CDIs. It is the most common cause of hospital-acquired infectious diarrhea, often leading to an increased

length of hospital stay as per studies from various parts of the developed world (Enoch & Aliyu, 2012). In the United States, *C. difficile* caused about half a million infections and 29,000 deaths (Lessa et al., 2015). Whereas, in Europe, it is estimated that about 152,905 CDI cases and 8382 CDI-associated deaths occur every year (Wiuff et al., 2018). The recent emergence of the highly virulent type pathogen BI/NAP1/027 is thought to increase incidences of CDI. Also, the severity of the infection and associated mortality have been reported not only in hospitals but also in facilities providing medical and non-medical supports and services to elderly people, where residents are at high risk of CDI for advanced age, previous hospitalization, and exposure to antibiotics (Jump et al., 2018; Karanika et al., 2017; Ricchizzi et al., 2018). The American Medical Association's Journal of Internal Medicine estimates that at the current rates of *C. difficile*, an additional $1.5 billion adds to the annual cost of health care. We should highlight the fact that *C. difficile* disproportionately affects older patients, where Medicare pays for 68 percent of all *C. difficile*-related hospital stays. Since 2013, the CDC has classified *C. difficile* as "an urgent threat," a designation for threats with the potential to become widespread and therefore require urgent attention to identify and prevent transmission of infection (Centers for Disease Control and Prevention, 2013). Unfortunately, CDI prevention efforts are hampered by a lack of data to support optimal prevention methods, especially for endemic CDI, thus justifying this study.

1.4 Aim of the study

1- The primary aim is to identify the risk factors, the demographic determinants, and clinical symptoms of hospital associated CDI.

2- The secondary aim is to assess the relationship between *C. difficile* toxin production with the above identified variables.

2.0 Methods

This study was conducted in a single academic hospital located in Pittsburgh, PA, which is a part of a large university-affiliated medical system. We gathered patients' information using the electronic medical record (EMRs) of adult patients admitted to the hospital from May 2020 to April 22; we identified a study population of 178 patients. These people were diagnosed with CDI as per our criteria (see Table 1). Cerner Power Chart software (Cerner Corp., Kansas City, MO) was used for medical record abstractions, and the TheraDoc (TheraDoc, Charlotte, NC) system was used for positive patient identification.

For this study, we collected the following details about CDI-positive patients: (a) sex, (b) age, (c) race, (d) HA-CDI, (e) use of antibiotics, (f) use of proton pump inhibitors (PPIs) during hospital admission, (g) use of laxative, (h) CDI-positive test date, and (i) clinal examination of CDI positive patients during of the period of the study.

For the age group, we separated the patients between more than sixty years or less than sixty years old. The patients were diagnosed as CDI positive if there were evidence of *C. difficile* toxin-producing bacteria, toxin-presence, or positive results from a confirmatory PCR toxin assay. In PCR testing, the CT or cycle threshold value is used, which represents the number of cycles needed to amplify the target DNA to a detectable level. The CT value determines the presence and severity of C. difficile infection. A low CT value indicates a higher bacterial load and may suggest an active infection. A high CT value, on the other hand, may suggest a lower bacterial load and indicate previous colonization or infection. Clinical symptoms were also matched with *C. difficile-positive* patients. For example, diarrheal symptoms were verified during *C difficile* testing. Patients were considered to have diarrhea if they had at least three unformed bowel movements recorded

in the electronic health record (EHR) within 24 hours on the day of or before sample collection. Some of the other criteria that we used for the study were (Table 1): (i) whether the patient took laxatives within three days prior to *C. difficile* testing, (ii) a lab test for the highest number of WBC within three days or 72 hours before and after testing the CDI, (iii) whether the patient took tube food within three days before *C. difficile* testing, (iv) whether the patient took a proton pump inhibitor within three days before *C. difficile* testing; (v) detailed histories of patients' use of antibiotic use within 30 days in all the patients; and (vi) *C. difficile* therapy within three days after the test results.

Table 1: Criteria for data collection.

Time points of usage prior to *C. difficile* testing	
Laxative	Within three days
Antibiotics	Withing 30 days
PPI	Within three days
Abdominal Pain	Within three days
Diarrhea / loose stool	Within three days
Fever > 38 ºC	Within three days
High WBC	Within three days
Time points after *C. difficile* test results	
***C. difficile* Treatment**	Within three days

3.0 Results

3.1 Medication and therapies as risk factors for *Clostridioides difficile*

In this study, we addressed whether prolonged medication and therapies such as the use of antibiotics, feed tubes, and proton pump inhibitors act as risk factors for *Clostridioides difficile* occurrences. We used relevant data from 124 patients. In **Figure 1**, we show a comparison of the risk factors.

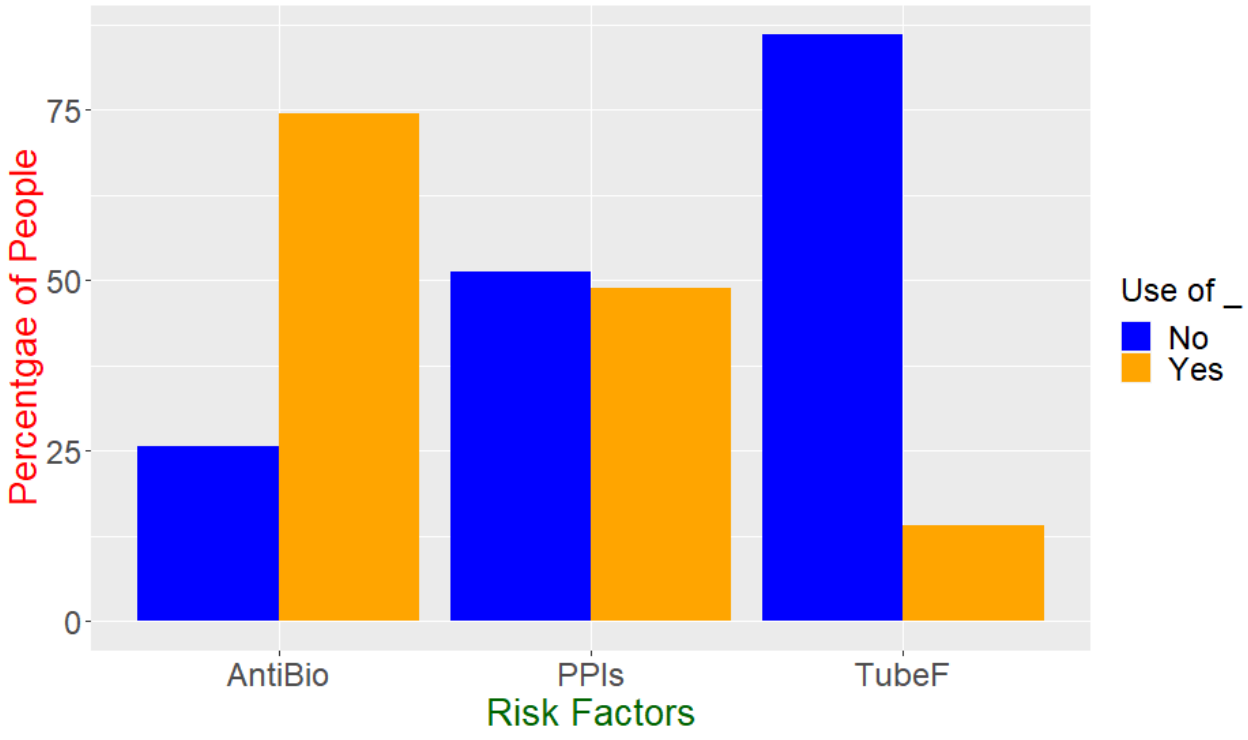

Figure 1: Proportion of *Clostridioides difficile* infected patients in percentage that were on medication and therapies namely – Antibiotics (AntiBio), Feed tubes (TubeF) and Proton pump inhibitors (PPIs).

3.1.1 Use of antibiotics

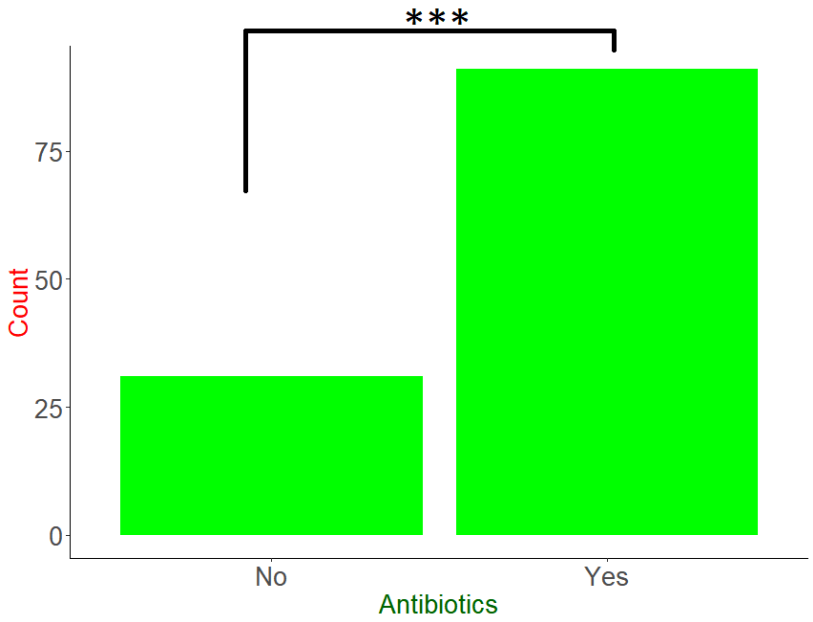

Figure 2: Incidences of *Clostridioides difficile* in patients on antibiotics, which is significantly greater. P < 0.0001 (***), 1-sample proportions test.

First, we tabulated the use of antibiotics by *C. difficile* patients. **Figure 2** depicts the proportion of patients who extensively used antibiotics and were infected with *C. difficile*. Based on analysis of the data, we are 95% confident that the population proportion of individuals with extensive use

Table 2: Medication and therapies as risk factors and their 1-sample propportions.

Risk factors of medication and therapies	1-sample proportions test p-value (users vs. non-users)
Antibiotics	< 0.0001 (***)
Proton pump inhibitors	0.8
Tube feed	< 0.0001 (***)

Table 3: Medication and therapies as risk factors and their 2-sample proportions.

Risk factors	p-value: 2-sample test for equality of proportions for Toxin +ve vs. -ve	p-value: 2-sample test for equality of proportions for CT values <25 vs. >25
Antibiotics	**0.96**	**0.80**
Proton pump inhibitors	**0.63**	**0.67**
Tube feed	**0.95**	**0.69**

of antibiotics that were infected with *C. difficile* is between 65.8% and 81.8%. Using a one-proportion z test, the proportion of patients who were *C. difficile* positive with extensive use of antibiotics is more significant than 50% occurred with a probability of $p = 9.2x10^{-8}$ (**Table 2**). The above analysis suggests that CDI occurs more readily in patients that extensively use antibiotics. Further analysis of the two-proportion z-test for the difference between toxin-positive patients who

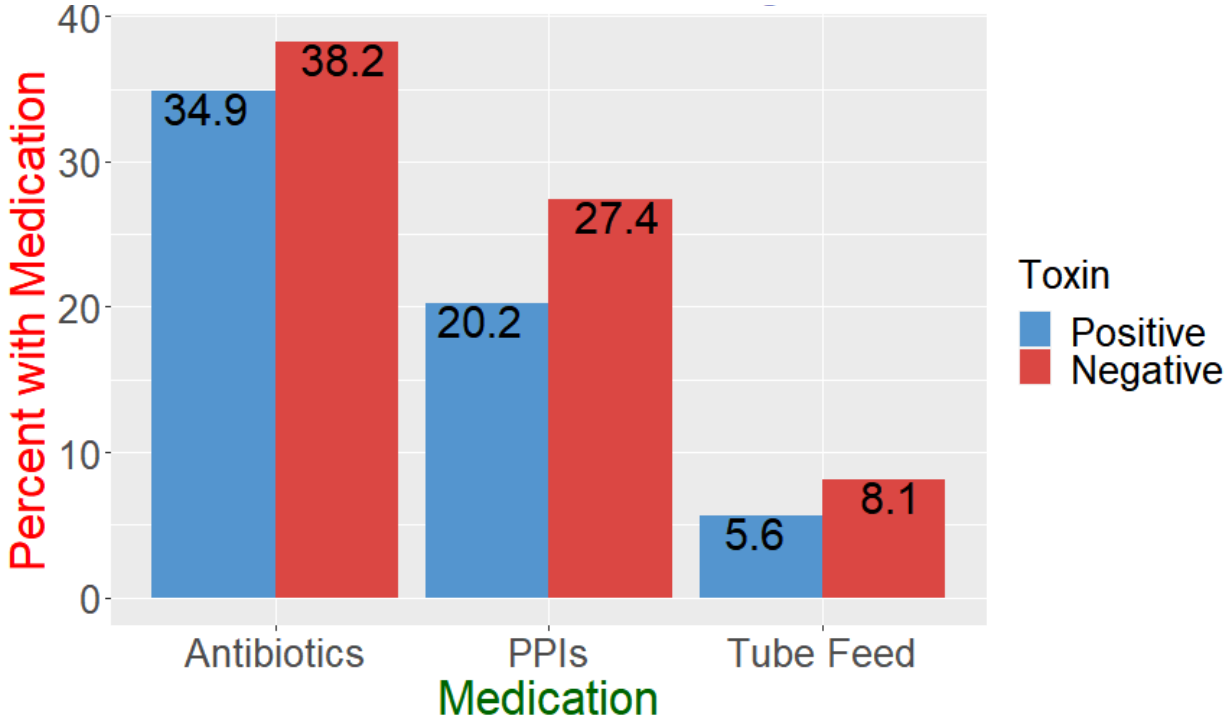

Figure 3: Percentage of *C. difficile* patients who were on Antibiotics, Proton pump inhibitors and feed tubes and were either toxin positive (Blue) or toxin negative (Red).

used antibiotics (34.9 %, **Figure 3**) and toxin negative patients who used antibiotics (38.2 %, **Figure 3**) resulted in a p-value of p=0.96 (**Table 3**), suggesting that there is not enough evidence to conclude that there is a statistically significant difference between the group. The two-proportion z-test between patients with CT < 25 and on antibiotics (16.4%, **Figure 4**) and patients with CT > 25 and on antibiotics (41.8%, **Figure 4**) is also non-significant (p= 0.80, **Table 3)**.

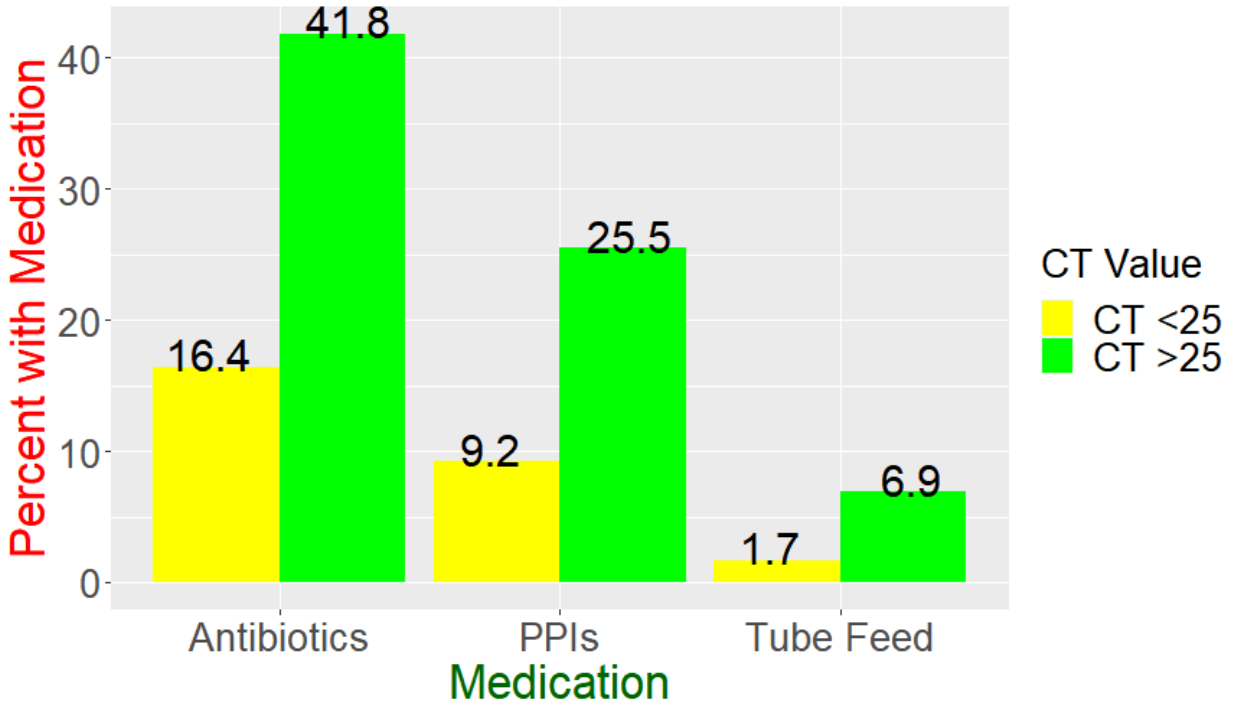

Figure 4: Percentage of *C. difficile* patients who were on Antibiotics, Proton pump inhibitors and feed tubes and had CT <25 (Yellow) or CT >25 (Green).

3.1.2 Use of proton pump inhibitors

We also looked at how the proton pump inhibitors (PPIs) acted as risk factors for *Clostridioides difficile.* In **Figure 5**, we plot the proportion of patients that did not use or use PPIs. The proportion of *C. difficile* patients who did not use or used PPIs were similar. The 95% confidence interval estimates that the proportion of PPI usage was between 39.5% to 57.2%, which contains the halfway mark. The estimated one-proportion z test also suggests that the probability that the proportion is different than 50% is p = 0.78, a non-significant value. Expectedly, a two-proportion z-test for the difference between toxin-positive patients who used PPIs (20.2%, **Figure**

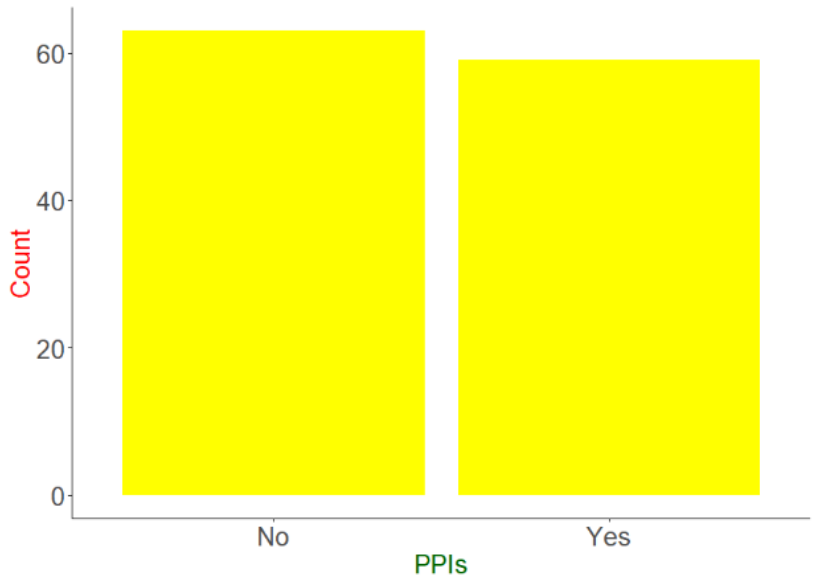

Figure 5: *Clostridioides difficile* incidences are similar between patients on or not on proton pump inhibitors. P = 0.78, 1-sample proportions test.

3) and toxin negative patients who used PPIs (27.4%, **Figure 3)** resulted in a p-value of p=0.6216 (**Table 3**), suggesting that there is not enough evidence to conclude that there is a statistically significant difference between the group. The two-proportion z-test between patients with CT < 25 (9.2%, **Figure 4**) and on PPIs and patients with CT > 25 (25.5%, **Figure 4**) and on PPIs is also non-significant (p= 0.67, **Table 3**).

3.1.3 Use of feed tubes

Similarly, we depict the use of feed tubes by *C. difficile* patients as shown in **Figure 6.**. The proportion of patients who extensively used tube feeds was small in numbers. The estimate of confidence interval with a 95% confidence level, the usage lies between 7.8% to 20.1%, which is much lower than half the patients. The one-proportion z test also suggests that the probability that

the proportion is less than 50% is p = 3.4×10^{-15} (**Table 2**), a highly significant value. Thus, *C. difficile* infected do not extensively use tube feeds. A two-proportion z-test for the difference between toxin-positive patients who used tube feeds (5.6%, **Figure 3)** and toxin-negative patients

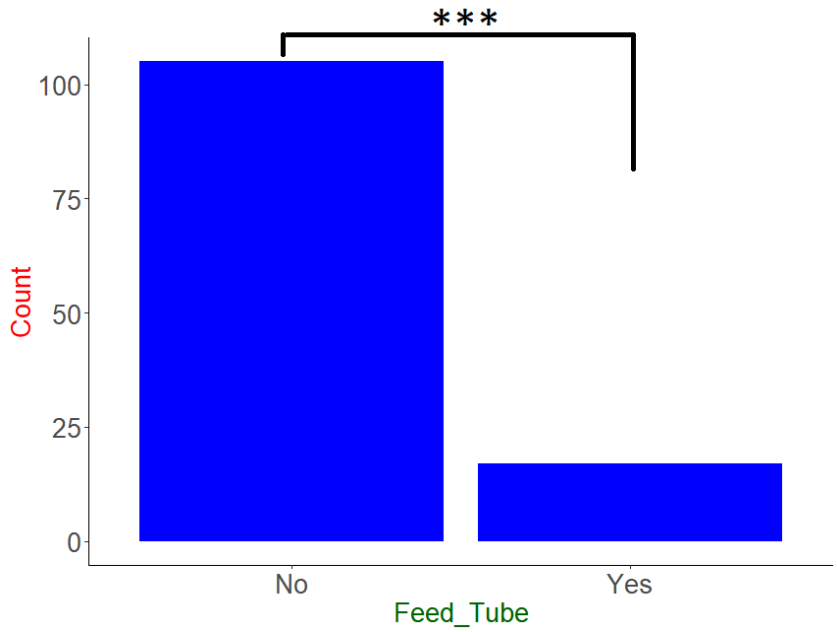

Figure 6: *Clostridioides difficile* incidences are significantly greater in patients not on tube feed. P < 0.0001 (***), 1-sample proportions test.

who used tube feeds (8.1%, **Figure 3**) yielded a p-value of 0.9 (**Table 2**), meaning there is not sufficient evidence to conclude there is a statistical difference between the group that are toxin positive patients and use tube feeds and the toxin negative patients who used tube feeds. Similarly, there is no sufficient evidence to conclude there is a statistical difference between the group that have a CT > 25 (6.9%, **Figure 4**) and use tube feeds and the patients who have CT < 25 (1.7%, **Figure 4**) and used tube feeds (p = 0.67, **Table 3**).

3.2 Demographic determinants of *Clostridioides difficile* incidences: age, gender, and race

We analyzed the association of CDI with the demographic factors – age, gender, and race – for this group, where age could be an extrinsic factor and gender and race could provide clues to intrinsic risk factors.

For this study, we had relevant demographic data for 124 patients. In **Figure 7,** we plot the distribution of the demographic factors such as age, gender, and race among the patients infected with *Clostridioides difficile*. The first bar shows that 73.4 % of the patients who were admitted with the infection who are older than 60 years. From now onwards, we will refer to the population older than 60 years as elderly. Our data thus indicate that about three-fourths of *Clostridioides difficile* incidences happened in the elderly. The second bar in the figure shows the percentage of 49.2% of the patients are males. This suggests that the patient population is roughly divided equally among males and females, suggesting that *Clostridioides difficile* incidences are not gender biased. The last bar shows 74.2 % of the patients are white population, the rest are non-whites. From our data, we thus see that *Clostridioides difficile* incidences predominantly occurred in the white population.

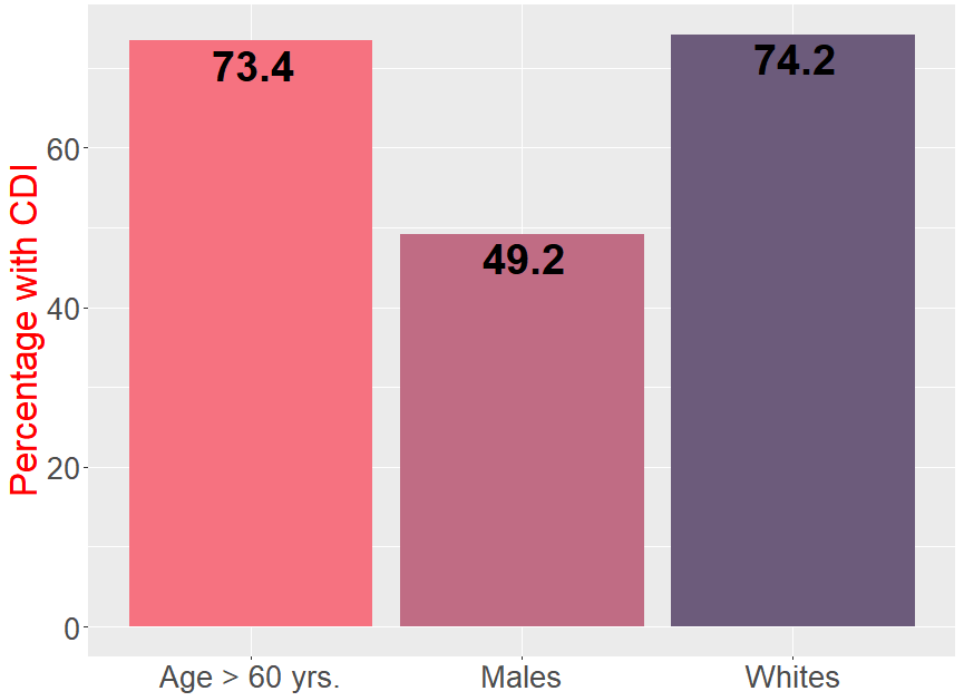

Figure 7: Proportion of CDI patients in the sample (n = 124) according to age (older than 60 yrs. vs. younger); Gender (Male vs. Female) and Race. (White vs. non-White).

3.2.1 Age-specific incidences

Similarly, we analyzed whether the elderly (>60 years) population in our sample has a significantly greater chance of contracting *Clostridioides difficile*. In **Figure 8**, we separately depict the proportion of elderly patients in terms of the number of patients who were infected with *C. difficile* as compared to the younger ones. Using analysis for confidence interval, we are 95% confident that the population proportion of individuals older than 60 years that were infected with

C. difficile is between 65.6% and 81.1%. This confidence stems from knowing that 95% of all confidence intervals generated by this procedure would succeed in capturing the actual value of

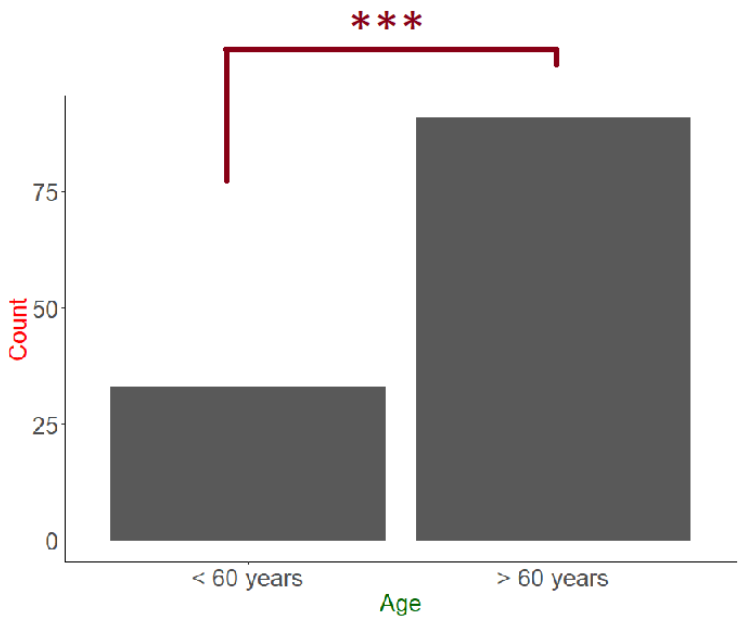

Figure 8: *Clostridioides difficile* incidences are significantly greater in patients older than 60 yrs. P < 0.0001 (***), 1-sample proportions test.

Table 4: Evaluation of demographic factors and their 1- and 2-sample proportions.

Demographic Factors	Proportions	1-sample proportions test p-value	2-sample test for equality of proportions for Toxin positives vs. negatives	2-sample test for equality of proportions for CT values < 25 vs. > 25
Age: ≥60 yrs. vs. <60 yrs.	91 : 33	< 0.0001 (***)	0.83	0.30
Gender: Male vs. Female	61 : 63	0.93	0.77	0.79
Race: White vs. Others	92 : 32	< 0.0001 (***)	0.27	0.04 (*)

the population proportion. Using a one-proportion z test under which the proportion of patients who were *C. difficile* positive are older than 60 years to be equal to 50% resulted in the rejection of the null hypothesis, thus suggesting that there was sufficient evidence to support the alternative hypothesis such that the proportion of elderly patients was more than 50% occurred with a probability of $p = 3.1 \times 10^{-7}$ (**Table 4**). Thus, as expected, the above analysis suggests that the elderly population has a higher chance of being infected. In the given population, our results confer with others that age is a risk factor for *Clostridioides difficile* incidences.

To test whether toxin-positive and toxin-negative patients differ in proportion among the elderly, we evaluated the two-proportion difference between the age groups. 34.1% of elderly patients were toxin-positive (**Figure 9**), and 39.0% of elderly patients were toxin-negative (**Figure 9**). The result from the two-proportion z-test for the difference between the two groups yielded a

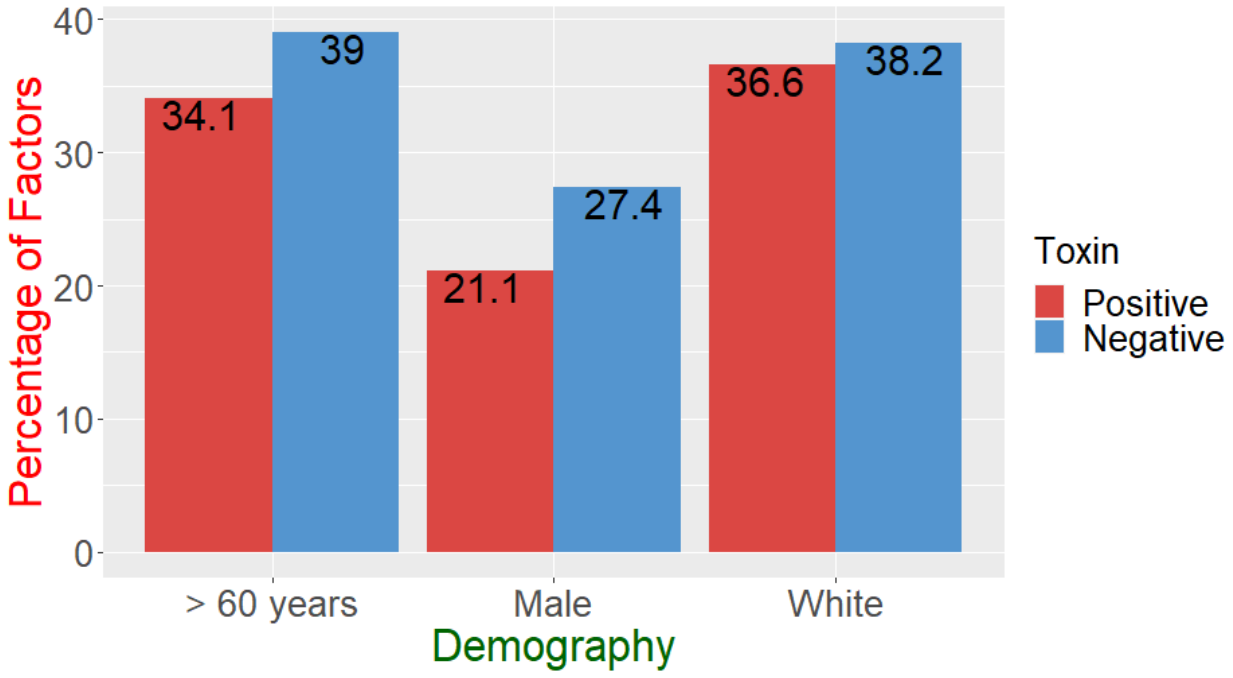

Figure 9: Percentage of *C. difficile* patients who were elderly (> 60 years), males and whites that were either toxin positive (Red) or toxin negative (Blue).

p-value of 0.83 (**Table 4**), suggesting that the data does not provide sufficient evidence to conclude there is a significant difference in the proportion of elderly patients who were toxin-positive with the group that was toxin negative. Similarly, to test whether CT values are different with age, we evaluated the two-proportion difference between the CT values in old age. 15.0% of elderly patients exhibited a CT value less than 25 (**Figure 10**), and 43.7% of the same patients exhibited a CT > 25 (**Figure 10**). The two-proportion z-test for the difference between the two groups yielded a p-value of 0.30 (**Table 4**), suggesting that the data does not provide sufficient evidence to conclude that there is a significant difference in the proportion of elderly patients who exhibited a CT < 25 with the elderly patients that exhibited a CT >25.

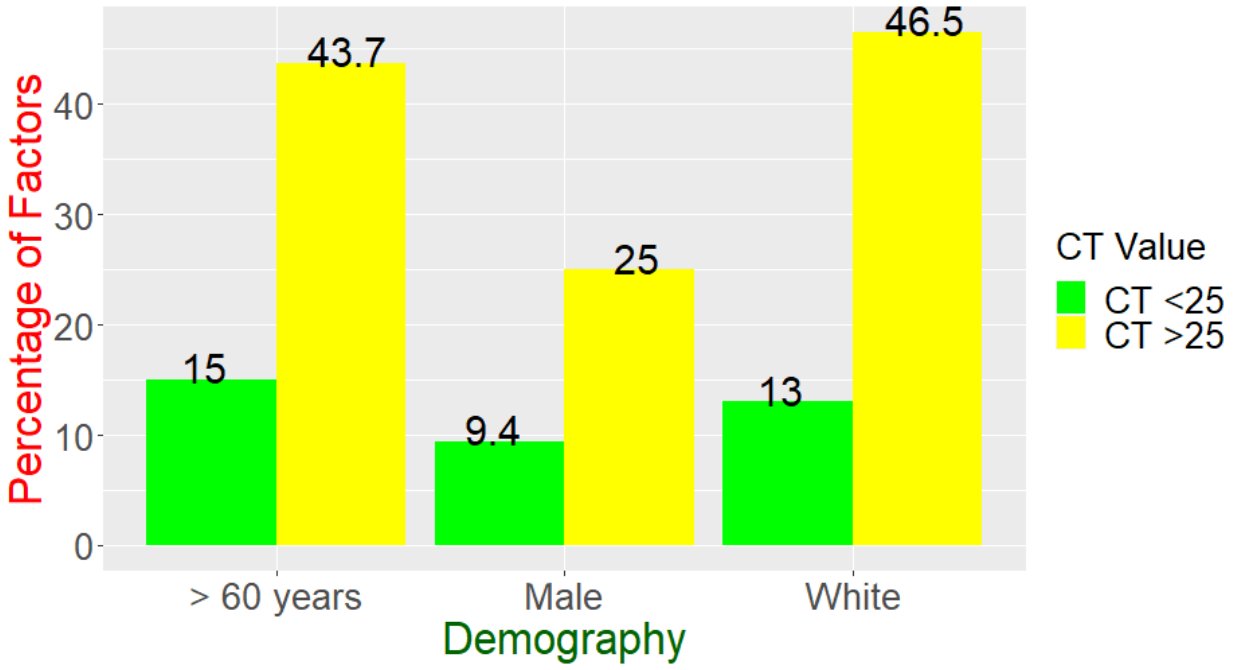

Figure 10: Percentage of *C. difficile* patients who were elderly (> 60 years), males and whites who had either CT <25 (Green) or CT >25 (Yellow).

3.2.2 Gender-specific incidents

We next asked whether any specific gender has a greater chance of contracting *Clostridioides difficile*. Data suggest that *C. difficile* infected gender equally, as depicted in **Figure 11**. A similar analysis for confidence interval, with 95% confidence, resulted in the population proportion of males infected with *C. difficile* between 40.3% and 58.0%. The one-proportion z test

Figure 11: *Clostridioides difficile* incidences occur in similar proportions between male or female population. P = 0.93, 1-sample proportions test.

under which the proportion of male patients who were *C. difficile* positive to be equal to 50% resulted in a non-significance (p = 0.93, **Table 4**). We found similar non-significant results when analyzing for 1-sample proportions for females. Additionally, to check by any chance whether the toxin-positive patients express differently with gender, we evaluated the two-proportion difference between the toxicity in the male gender. 21.1% of males were toxin-positive (**Figure 9**) and 27.4 % of males were toxin-negative patients (**Figure 9**). The result from the two-proportion z-test for the difference between the two groups yielded a p-value of 0.77 (**Table 4**), suggesting that the data does not provide sufficient evidence to conclude there is a significant difference in the proportion of males who were toxin-positive and the group of males that were toxin negative. Similarly, to test whether toxin-positive patients express differently with gender, we evaluated the two-proportion difference between the CT values (<25 or >25) in the male gender. 9.4% of males exhibited a CT value < 25 (**Figure 10**), and 25.0% of the same patients exhibited a CT > 25. The

two-proportion z-test for the difference between the two groups also yielded a non-significant p-value (p = 0.79, **Table 4**). Not to mention, we found similar non-significant results when analyzing for 2-sample proportions when considering females.

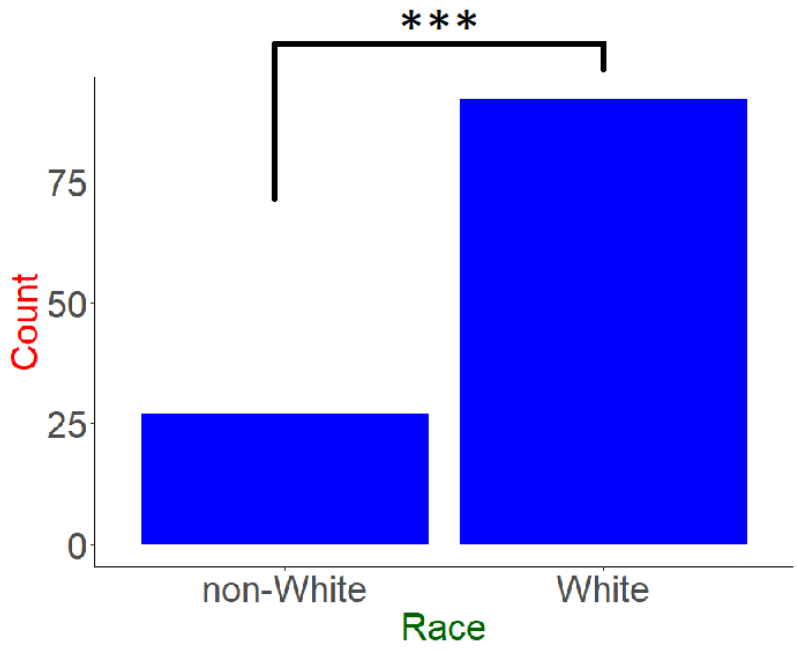

Figure 12: *Clostridioides difficile* incidences are significantly greater in white patients compared to non-whites. P < 0.0001 (***), 1-sample proportions test.

3.2.3 Race-specific incidences

We also compared the occurrence of *Clostridioides difficile* in the white population with the non-white race, as plotted in (**Figure 12).** Unforeseeably, the white patients exhibited a higher occurrence of the infection. The 95% confidence interval estimates that the white population has a greater chance ranging between 69.8% to 84.8%, to be infected with *Clostridioides difficile* as compared to the non-white population. The one-proportion z test under which the proportion of white patients who were *C. difficile* positive to be equal to 50% resulted in a significant p-value (p

= 4.4 x 10^{-9}, **Table 4**). Similarly, 80.3% of the white population were toxin-positive patients, and 70.1% of toxin-negative patients were whites. The result from the two-proportion z-test for the difference between toxin-positive white patients (36.6%, **Figure 12**) and toxin-negative white patients (38.2%, **Figure 12**) yielded a p-value of 0.27 (**Table 4**), suggesting that the data does not provide sufficient evidence to conclude there is a significant difference in the proportion of white patients who were toxin-positive and the group of the white population that were toxin negative. 13.0% of whites exhibited a CT value < 25 (**Figure 10**), and 46.5% of the same patients exhibited a CT > 25 (**Figure 10**). Surprisingly, the two-proportion z-test for the difference between the two groups also yielded a barely significant p-value (p = 0.045, **Table 4**). Thus, the result from a two-proportion z-test for the difference between the two groups suggests that there is a statistically significant difference in the proportion of whites who exhibit a CT value < 25 and the proportion of whites who exhibit a CT value > 25.

In a short summary of the demographic analysis, we found that old age and race might act as demographic risk factors for CDI. From our analysis, the proportion of elderly patients is significantly greater than that of patients younger than 6o years. Thus, *Clostridioides difficile* disproportionately affects the elderly, suggesting old age is a significant risk factor for the infection. Since *Clostridioides difficile* occurs equally among males and females, gender may not be a risk factor for *Clostridioides difficile*, at least in our study. Unexpectedly, the white population was infected with *Clostridioides difficile* in greater proportions as compared to the non-white population suggesting *Clostridioides difficile* can disproportionately affect a race. Hence race could be a risk factor for CDI. Further evaluation of the demographic factors suggests that the demographic proportion is similar between toxin positive and toxin negatives. For the same reason, the proportion of whites who exhibit a CT value greater than 25 is different than the proportion of whites who exhibit a CT value less than 25 and is statistically significant.

3.3 Clinical symptoms of *Clostridioides difficile*

For this study, we analyzed the pattern of clinical symptoms in CDI patients. we addressed whether the patients exhibited the known clinical symptoms reported in the literature, such as fever, abdominal pain, cramping, and tenderness. As in the above sections, we used relevant data from 124 patients to determine the clinical symptoms.

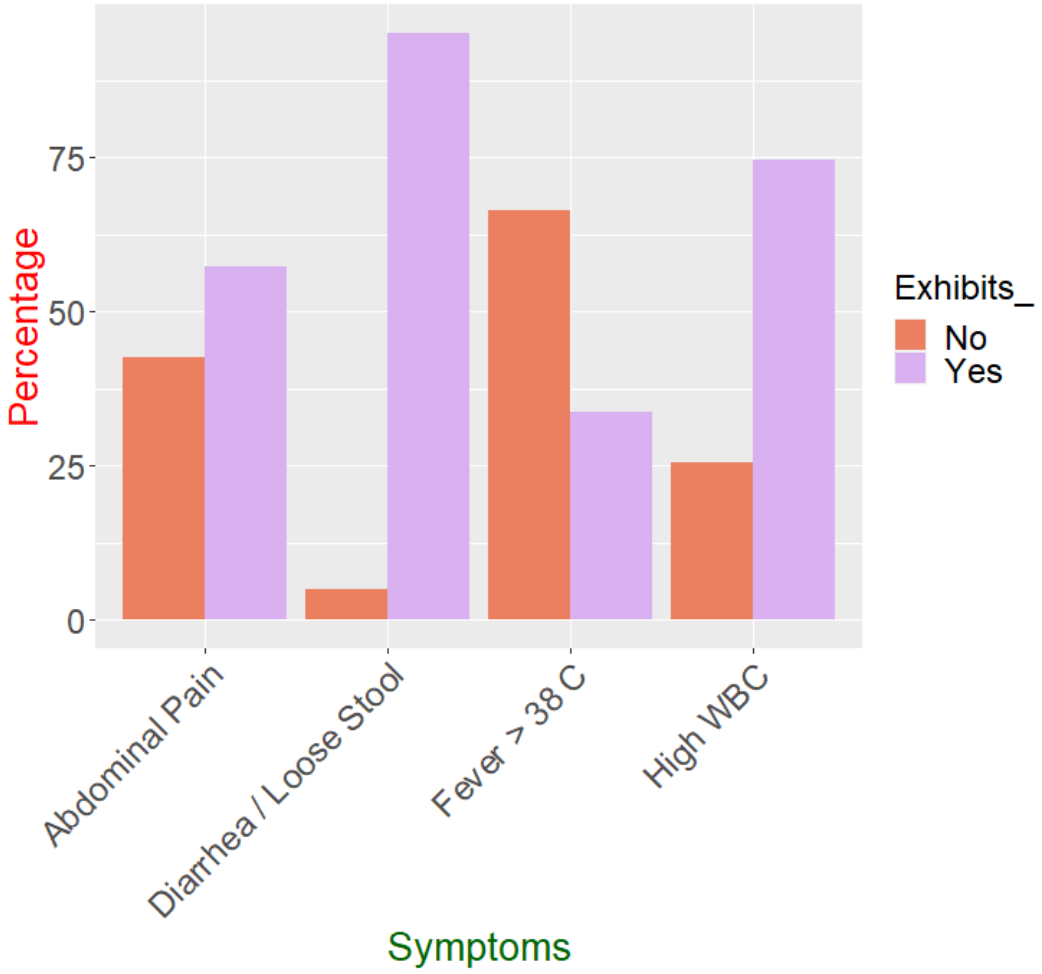

Figure 13: Proportion of CDI patients in the sample (n = 124) exhibiting clinical symptoms such as (1) abdominal pain / cramping, tender distension, (2) diarrhea / loose stool, (3) fever > 38 oC and (4) High WBC.

In **Figure 13** we show the clinical symptom proportions such as (1) abdominal pain / cramping, tender distension, (2) diarrhea / loose stool, (3) fever > 38 ^{o}C, and (4) High WBC, among the patients infected with *Clostridioides difficile*. The first pair of bars shows that 57.4 % of the patients who were admitted with the infection exhibited abdominal pain, cramping, and/or tender distension. As expected, the second bar pair in the figure shows that a very high percentage of the patients (95%) had diarrhea or loose stool. The third bar pair shows that one-third, 33.6% to be precise, of the CDI patients, exhibited body temperature greater than 38 ^{o}C. The last bars show 74.6 % of the patients had a high WBC count. We can probably use these data to evaluate the clinical symptoms in *Clostridioides difficile* patients.

3.3.1 Patients with abdominal pain, cramping and tender distension

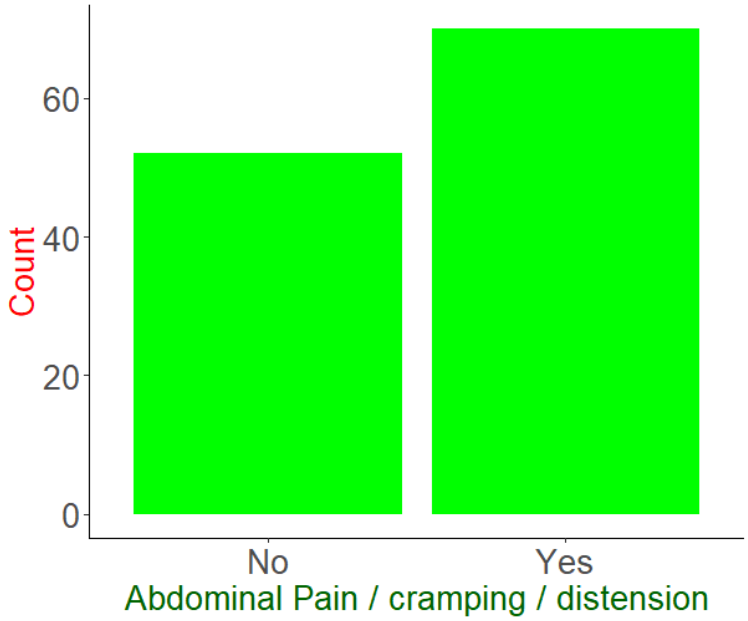

Figure 14: *Clostridioides difficile* patients exhibiting abdominal pain, cramping and/or tender distension. P = 0.1, 1-sample proportions test.

First, we tabulated whether *C. difficile* patients exhibit abdominal pain, cramping and/or tender distension. **Figure 14** depicts the proportion of patients who had the symptoms and were infected with *C. difficile*. Based on analysis of the data, we are 95% confident that the population proportion of individuals with the symptoms that were infected with *C. difficile* is between 48.1% and 66.2%.

Table 5: 1-sample proportions of patients with clinical symptoms.

Clinical symptoms of CDI	1-sample proportions test p-value (No vs. Yes)
Abdominal pain / cramping / tender distension	0.1
Diarrhea / loose stool	< 0.0001
Fever > 38 ºC	0.0004
High WBC	< 0.0001

Table 6: 2-sample propportions of patients with clinical symptoms.

Clinical symptoms of CDI	p-value: 2-sample test for equality of proportions for Toxin +ve vs. -ve	p-value: 2-sample test for equality of proportions for CT values <25 vs. >25
Abdominal pain / cramping / tender distension	1	1
Diarrhea / loose stool	0.8	0.4
Fever > 38 ºC	0.05	0.8
High WBC	0.2	1

Using a one-proportion z test, the proportion of *C. difficile* patients were similar between those who had the symptom and those who did not (p =0.1; **Table 5**).

The above analysis suggests that *C. difficile* infected patients may exhibit abdominal pain or not with equal probability. Further analysis of the two-proportion z-test for the difference between toxin-positive patients who had the symptoms (26.2 %, **Figure 15**) and toxin negative patients who had the symptoms (30.9 %, **Figure 15**) resulted in a p-value of p = 1 (**Table 6**), suggesting that there is not enough evidence to conclude that there is a statistically significant difference between the group. The two-proportion z-test between patients with CT < 25 and with the symptoms (13.3 %, **Figure 16**) and patients with CT > 25 and with the symptoms (28.9 %, **Figure 16**) is also non-significant (p= 1, **Table 6**).

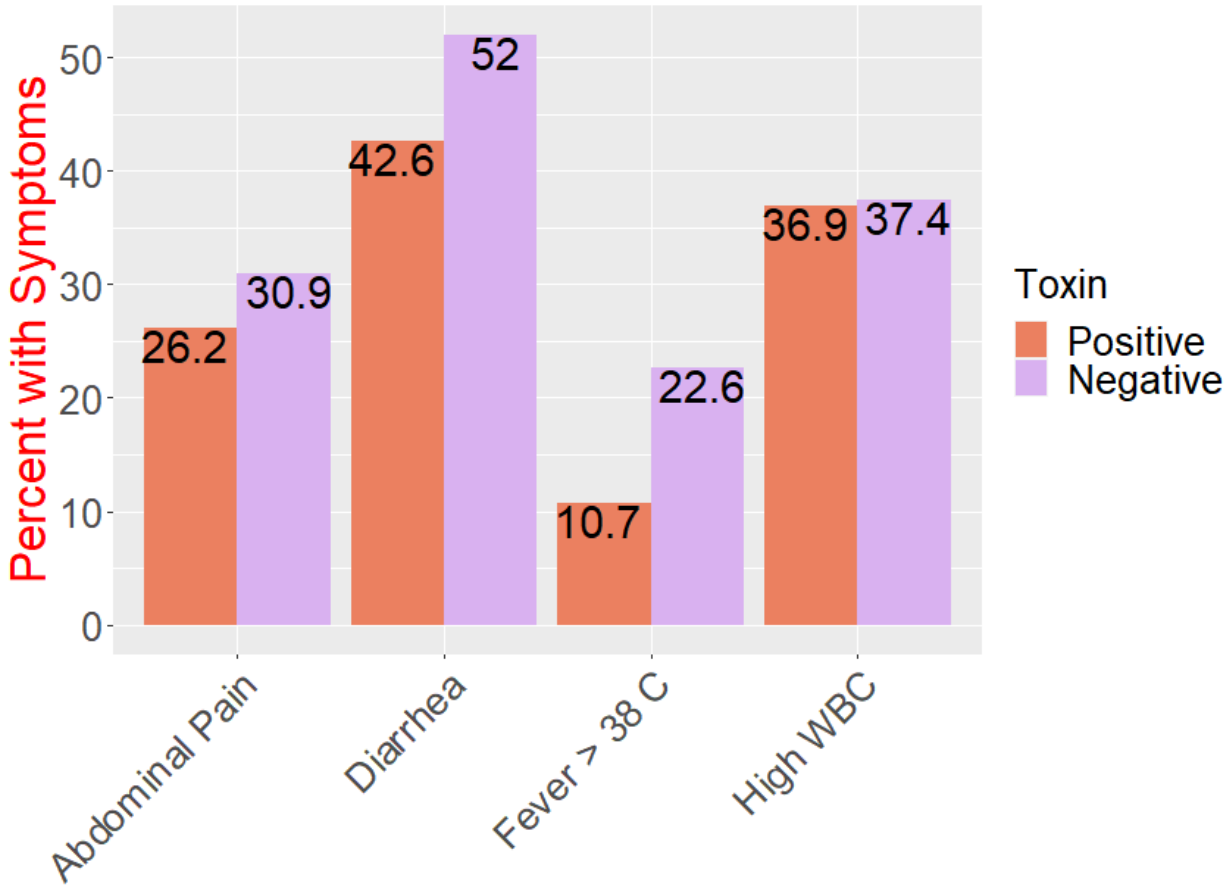

Figure 15: Percentage of *C. difficile* patients with clinical symptoms and were toxin positive (red) or toxin negative (blue).

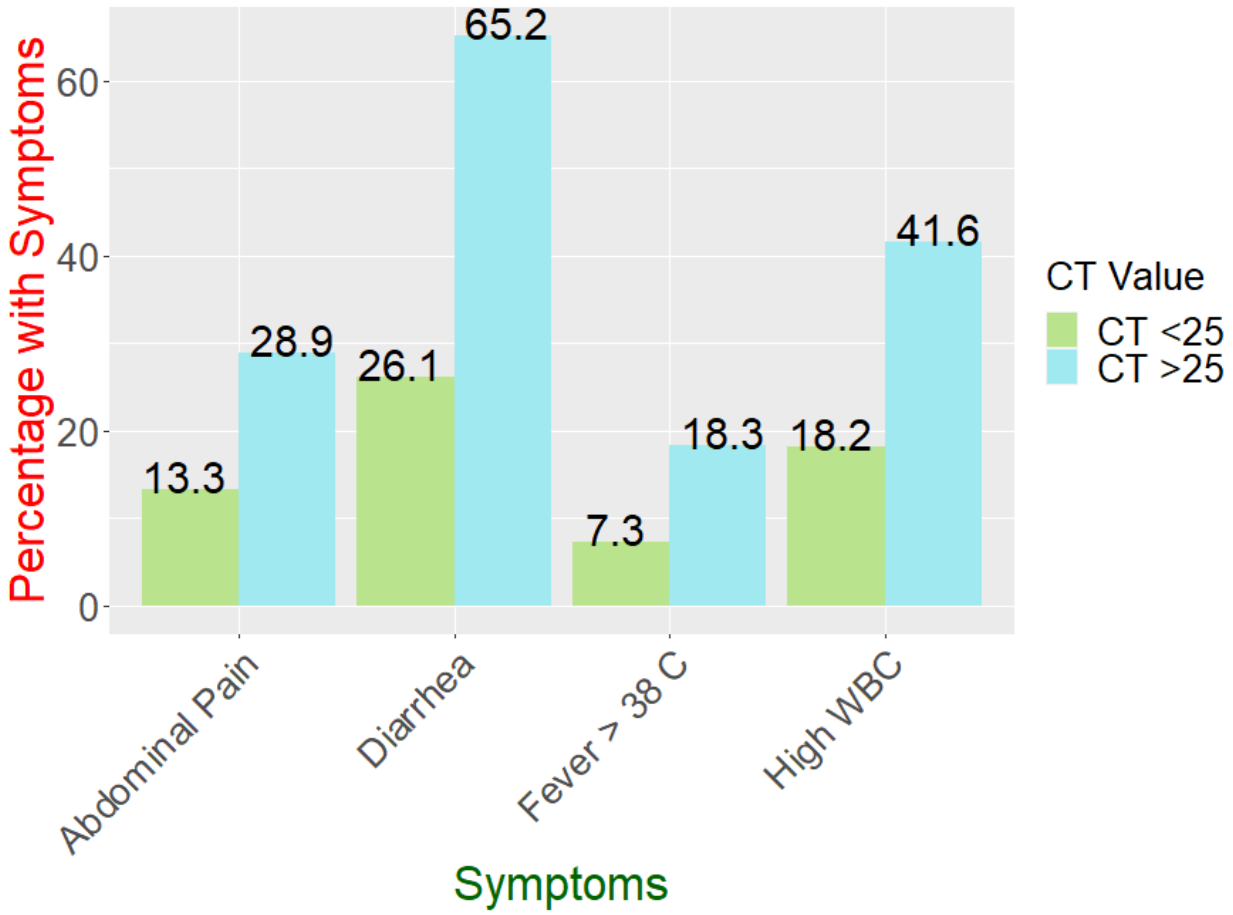

Figure 16: Percentage of *C. difficile* patients with clinical symptoms and had CT <25 (green) or CT >25 (blue).

3.3.2 Patients with diarrhea / loose stool

We also looked at whether the *Clostridioides difficile* patients exhibited diarrhea or loose stool. In **Figure 17**, we plot the proportion of patients who had the symptoms. A significantly greater proportion of *C. difficile* patients had the symptoms. The 95% confidence interval estimates

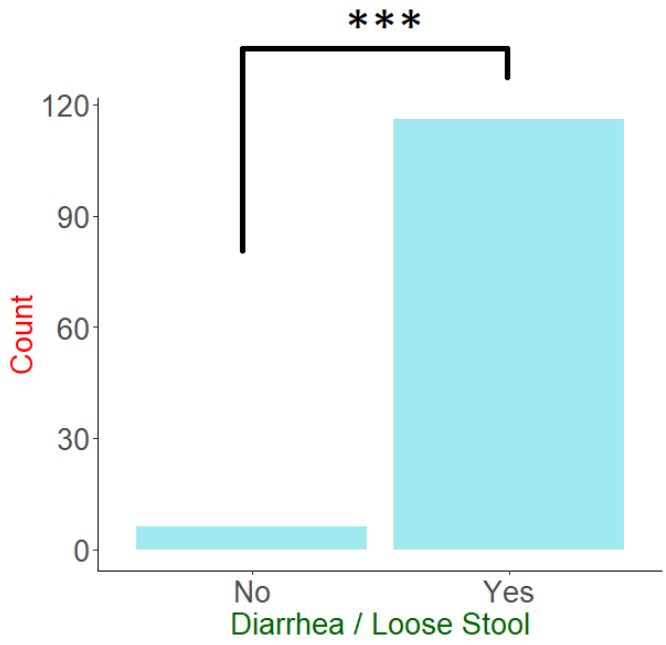

Figure 17: *Clostridioides difficile* incidences of diarrhea / Loose stool are in very significantly greater proportions. P < 0.0001, 1-sample proportions test.

that the proportion of diarrhea patients was between 89.1 % to 98.0 %. The estimated one-proportion z test also suggests that the probability that the proportion is different than 50% is p < 0.0001, expectedly a highly significant value. However, a two-proportion z-test for the difference between toxin-positive patients who had the symptom (42.6%, **Figure 15**) and toxin negative patients who had the symptom (52.0, **Figure 15**) resulted in a p-value of p=0.8 (**Table 6**), suggesting that there is not enough evidence to conclude that there is a statistically significant difference between the group. The two-proportion z-test between patients with CT < 25 (26.1%, **Figure 16**) and with the symptom and patients with CT > 25 (65.2%, **Figure 16**) and with the symptom is also non-significant (p= 0.4, **Table 6**).

3.3.3 Patients with fever > 38 ºC

Similarly, we depict the number of *C. difficile* patients with fever as shown in **Figure 18**. The proportion of patients who had a fever > 38 ºC was smaller in numbers compared to those who had less. The estimate of confidence interval with a 95% confidence level, the usage lies between 25.5% to 42.8%, which is about one-third of the patients. The one-proportion z test also suggests that the probability that the proportion is less than 50% is p = 0.0004 (**Table 5**), a very significant value. Thus, *C. difficile*-infected patients do not exhibit high fever. A two-proportion

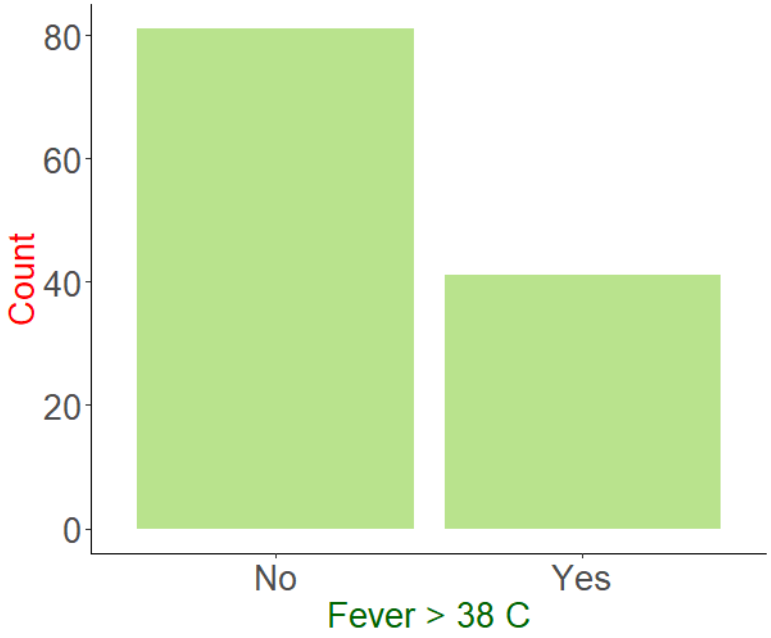

Figure 18: *Clostridioides difficile* patients with fever are significantly low. P = 0.0004 (***), 1-sample proportions test.

z-test for the difference between toxin-positive patients who had a fever > 38 ºC (5.6%, **Figure 15**) and toxin-negative patients who had a fever > 38 ºC (8.1%, **Figure 15**) yielded a p-value of 0.05, which is very close to significance (**Table 6**), meaning there is barely not sufficient evidence to conclude there is a statistical difference between the group that is toxin positive patients and had a fever > 38 ºC and the toxin negative patients who had a fever > 38 ºC. However, there is no sufficient evidence to conclude there is a statistical difference between the group that has a CT > 25 (6.9%, **Figure 16**) and had a fever > 38 ºC and the patients who have CT < 25 (1.7%,**, Figure 16**) and had a fever > 38 ºC (p = 0.8,**Table 6**).

3.3.4 Patients with high WBC count

We also depict the number of *C. difficile* patients with high WBC count, as shown in **Figure 19**. The proportion of patients who had a high WBC count was greater in numbers compared to

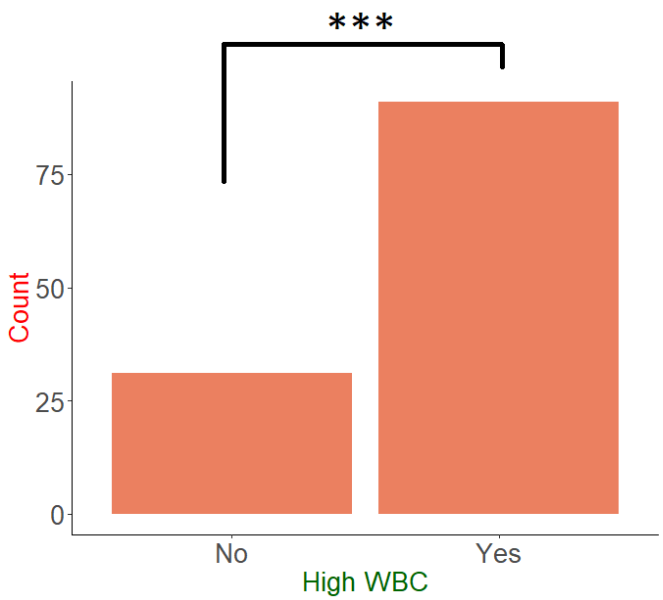

Figure 19: *Clostridioides difficile* patients with high WBC count are significantly greater. P < 0.0001 (***), 1-sample proportions test.

those who had low. The estimate of confidence interval with a 95% confidence level, the usage lies between 65.8% to 81.8%. The one-proportion z test also suggests that the probability that the proportion is greater than 50% is p < 0.0001 (**Table 5**), a very significant value. Thus, CDI patients' high WBC count is significantly greater. A two-proportion z-test for the difference between toxin-positive patients who had a high WBC count (36.9%, **Figure 15**) and toxin-negative patients who had a high WBC count (37.4%, **Figure 15**) yielded a p-value of 0.2, which is not significant (**Table 6**), meaning there is no sufficient evidence to conclude there is a statistical difference between the group that is toxin positive patients and had a high WBC count and the toxin negative patients who had a high WBC count. Similarly, there is no sufficient evidence to conclude there is a statistical difference between the group that has a CT < 25 (18.2%, **Figure 16**) and had a high WBC count and the patients who have CT > 25 (41.6%, **Figure 16**) and had a high WBC count (p = 1, **Table 6**).

4.0 Discussions

For this study, we gathered patients' information on adult patients admitted to one hospital, where we identified a study population of 178 patients. Although the known risk factors for CDI are previous hospitalization, underlying disease, advanced age, and, most importantly, the use of antibiotics (Smits et al., 2016), here, we focused on two broad areas in search of risk factors: demographically determinants and use of medication and therapies.

As demographic determinants, we selected age, gender, and race as possible risk factors. Advanced age is a known risk factor for CDI (Asempa & Nicolau, 2017; Czepiel et al., 2019; Loo et al., 2011; Smits et al., 2016). Our analysis suggests that the elderly population has a very significantly higher chance of being infectious to CDI as compared to their younger counterparts. However, the proportion of elderly patients who were toxin positive was similar in proportion to the elderly patients that were toxin negative. Similarly, the proportion of elderly patients who exhibited a CT > 25 and the same group that exhibited a CT <25 was similar. The result that elderly patients are highly disposed to the infection is well expected and also well supported by published work from around the world. According to a report from CDC, one-third of CDIs occur in patients 65 years or older (CDC, 2013). Based on a 15-month prospective Canadian cohort study, the authors found that for each additional year of age above 18 years, the risk of health care–acquired CDI increased by 2% (Loo et al., 2011). As people age, their immune system becomes less efficient at fighting off infections. This can make them more susceptible to *C. difficile*, which can colonize the gut more easily in older adults. Additionally, many older adults take multiple medications, which can also disrupt the gut bacteria, making them more susceptible to *C. difficile*. Also, older adults may have reduced mobility, making it difficult for them to maintain good hygiene and avoid

exposure to *C. difficile*. However, all these factors may be dependent on each, along with other factors acting as underlying confounders for old age susceptibility to CDI. According to studies performed in the European Union, it has been projected that the demographic old-age dependency ratio (the ratio of those aged >65 years old to those aged 15–64 years) will increase from 27.8 to 50.1% between 2013 and 2060 (Commission, 2014). Thus, the impact of CDI will become considerable in the coming years because in the US, the aged population is projected to increase from 13.7% to 20.9% between 2012 and 2050 (Ortman, J. M., Velkoff, V. A. & Howard, 2014). Thus, as our understanding of CDI continues to evolve, it is very well accepted by now that advanced age is a major risk factor and one that results in substantial morbidity and mortality (Asempa & Nicolau, 2017).

Next, we analyzed whether CDI-related hospitalization is gender specific. Unsurprisingly, we did not observe any clear differences in CDI by gender. Although there is a scarcity of data that separates the *Clostridioides difficile* infected patients by gender, nevertheless, in a published study about CDI-related hospitalization rates in Madrid (Spain) among 13,526 patients recorded from various hospitals and over a 12-year period, no gender difference was observed (Esteban-Vasallo et al., 2016). Thus, our results are similar to what has already been reported.

The last demographic factor that we analyzed was whether the CDI infection in our patients exhibited any race-specific relationship. Our results show that the occurrence of *Clostridium difficile* in the white population as compared to the non-white race was highly significantly greater. Our results thus show that in addition to increased age, the white race is also a distinctly predisposing factor for CDI, similar to the published report by Testore et al. (Killeen et al., 2014; Testore et al., 1986). According to another published analysis, including data from 7 US states encompassing a population of ∼10 million persons, showed that the white race seems to be at higher risk of CDI. Interestingly the study reports that whites had ∼50% higher incidence

compared with blacks and a 3-fold higher incidence compared with other nonblack races (Lessa et al., 2014). The reasons for white individuals to be at increased risk of CDI compared with other races are still unknown. However, researchers hypothesize that greater access to healthcare for the white population might increase the potential for more antibiotic exposure, or increased diagnostic testing may have a significant role in the differences in race-specific CDI incidence (Kullgren et al., 2010; Schappert, 1999).

Our results are also well supported by another retrospective study, where CDI risk factors were identified by comparing the demographic and clinical characteristics of Kaiser Permanente Northern California members that were ≥18 years. In the study, conducted from May 2011 through July 2014, 9986 CDI cases were identified, and individuals with CDI were more likely to be from the white race (70% vs 53%) than either the Black, Asian, or Hispanic population (Aukes et al., 2021).

Our results also showed that the proportion of white patients who were toxin positive was similar in proportion to the white patients that were toxin negative. However, the proportion of white patients who exhibited a CT > 25 was significantly greater than the same group that exhibited a CT <25. The reasons for the higher proportion of *Clostridium difficile*-infected white patients exhibiting CT > 25 compared to the same group exhibiting CT < 25 are still not clear. Of all patients with antibiotic-associated diarrhea, 20–30% is caused by *C. difficile* (Bartlett & Gerding, 2008). According to the literature, the most important known risk factor for CDI is the use of antibiotics (K. A. Brown et al., 2014; Czepiel et al., 2019; Leffler & Lamont, 2015; Smits et al., 2016; Stevens et al., 2011). Based on our analysis, the number of CDI patients who were on antibiotics was very significantly greater than the patients who were without the prescription. However, the antibiotic-prescribed patients who were toxin positive were similar in proportion to the antibiotic-prescribed patients that were toxin negative. Similarly, the proportion of antibiotic-

prescribed patients who exhibited a CT > 25 and the antibiotic-prescribed patients that exhibited a CT <25 was similar. Thus, our results show that the patients who were on antibiotics are highly disposed to the infection. This result is well expected and also well supported by various published reports. This was the case in a large cohort study of more than 200,000 hospitalized patients, where it was found that: the primary determinant of hospital-acquired CDI was the severity of illness and exposure to antibiotics (Forster et al., 2017). In another study, it was reported that each 10% increase in ward-level antibiotic exposure was associated with a 2.1 per 10 000 increase in *C. difficile* incidence (K. Brown et al., 2015). According to another study, among the three main factors to the development and severity of CDI, which are exposed to systemic antimicrobial therapy for other infections, exposure to *C. difficile* spores, and the host immune response, the risk of CDI is the highest during systemic antimicrobial therapy and in the first month after cessation of antimicrobial therapy thereafter. The same authors report that antimicrobials that pose the greatest risk of CDI are clindamycin, cephalosporins, and fluoroquinolones, and to a lesser frequency, macrolides and sulfonamides (Asempa & Nicolau, 2017). Similarly, in patients seeking medical attention for community-onset diarrheal illnesses at Yale–New Haven Hospital (New Haven, CT, USA), 3.9% were positive for *C. difficile* toxin, where most used antimicrobial drugs (Hirshon et al., 2011). More importantly, in a meta-analysis of forty-nine published reports that ranked individual antibiotic use in relation to the risk of CDI, it was found that exposure to an antibiotic was shown to be statistically significantly associated with CDI (Bignardi, 1998).

Table 7: Antibiotic classes and their association with CDI (adapted from Ref. (Leffler & Lamont, 2015)).

Clindamycin	Very common
Ampicillin	Very common
Amoxicillin	Very common
Cephalosporins	Very common
Fluoroquinolones	Very common
Other penicillin	Somewhat common
Sulfonamides	Somewhat common
Trimethoprim	Somewhat common
Trimethoprim–sulfamethoxazole	Somewhat common
Macrolides	Somewhat common
Aminoglycosides	Uncommon
Bacitracin	Uncommon
Metronidazole	Uncommon
Teicoplanin	Uncommon
Rifampin	Uncommon
Chloramphenicol	Uncommon
Tetracyclines	Uncommon
Carbapenems	Uncommon
Daptomycin	Uncommon
Tigecycline	Uncommon

All antibiotic classes have been associated with CDI, but clindamycin, cephalosporins and fluoroquinolones are cited most frequently in reports (Leffler & Lamont, 2015; Slimings & Riley, 2014) (see also Table 7). Paradoxically, many predisposing antibiotics act against *C. difficile*, but metronidazole has been reported to both incite the disease and provide effective treatment. In addition

to the antibiotic class, the number of administered antibiotics, dose and duration of therapy have been identified as risk factors for CDI (Smits et al., 2016). Conversely, the use of fluoroquinolones did not influence the risk of CDI for patients with previous hospital admission, based on a prospective case–control study at the Leiden University Medical Center, the Netherlands, during a period of 34 months (Hensgens et al., 2011).

After antibiotic therapy, the intestinal flora is disrupted that persists for more than 3 months. These conditions keep the patients susceptible to CDI for a long time (Smits et al., 2016). Simply, antibiotics can disrupt the balance of beneficial bacteria in the gut. This can happen in several ways. One way is by killing off a wide range of bacteria, including the beneficial ones that normally help to keep the gut healthy. This can create an imbalance in the gut microbiome, allowing harmful bacteria like *C. difficile* to overgrow and cause an infection. Additionally, antibiotics can change the environment of the gut, making it less hospitable to beneficial bacteria and more conducive to the growth of harmful bacteria. This can also contribute to the overgrowth of *C. difficile* and other harmful bacteria. Another way antibiotics can disrupt the balance of beneficial bacteria in the gut is by altering the metabolism of the gut bacteria. This can lead to the production of harmful by-products and toxins, which can cause inflammation and damage to the gut lining. This can in turn make the gut more susceptible to infections and other health problems. All these reasons can plausibly contribute to antibiotics as a risk factor for *C. difficile*.

PPI has been identified as an independent risk factor for developing CDI and associated diarrhea in the ICU (Barletta et al., 2013; Buendgens et al., 2014; Jayatilaka et al., 2007), though the mechanism is unclear. But not all available reports attest to this finding. As for example, in the meta-analysis of 23 studies assessing the relationship between PPI and CDI, it was found that fourteen studies identified a significant association between CDI and PPI, but the association was not statistically significant in the remaining nine studies (Arriola et al., 2016). However, using the relevant available literature, the authors calculated a pooled odds ratio of 1.81 for the association

between PPI use and the incidence of hospital-acquired CDI. In another study that evaluated the duration of therapy at which CDI risk increases, it was found that patients in whom CDI developed were more likely to have received a PPI and had more than 5 days duration of PPI therapy than those who did not have CDI development (Barletta et al., 2013). Similarly, Short course Proton pump inhibitors of 1-2 days heighten the risk and enhance hospital-acquired *Clostridium difficile* infection (HACDI). Also, PPIs combined with antibiotic therapy synergistically enhance the risk for HACDI (Wombwell et al., 2018). The heightened risk could continue for up to 28 days, as reported in this article (Seto et al., 2014). On the contrary, in a population-based database to identify individuals with ≥5 years of continuous PPI use along with non-PPI using controls, it was found that very long-term use of PPI does not manifest changes in their gut microbiome that would obviously pre-dispose to the development of CDI (Clooney et al., 2016).

Surprisingly, our data did not exhibit any clear differences in CDI by PPI use compared to those who did not. The exact mechanism by which PPIs lead to increased acquisition of *C. difficile* is not clear but appears to be multifactorial. Evidence exists linking PPIs to gastric pH increases resulting in bacterial overgrowth and increased spore survival (Wombwell et al., 2018). Additionally, evidence also exists for non-pH-related mechanisms such as alterations in neutrophil activity limiting bactericidal activity and enhancement of *C. difficile* toxin expression. It has been suggested that optimization of PPI use in the inpatient setting should be a focus of infection prevention programs. Minimizing inappropriate use may have a significant impact on rates of hospital-acquired CDI. This might be the case for the patients involved in our study, but it requires further verification.

There is evidence that conventional enteral tube feeding is associated with increased risk of CDI (O'Keefe, 2010). In a study that determined the incidence of *C. difficile* acquisition in tube-fed patients, it was found that more tube-fed patients acquired *C. difficile* compared to non-tube-

fed patients (Bliss et al., 1998). However, our results show that the proportion of CDI patients who extensively used tube feeds were much smaller in proportion to patients who did not. It is likely that this is a false negative result because it is common that patients who receive enteral nutrition who had a prolonged hospital stay prior to the *C. difficile* diagnosis, often in critical care areas where antibiotics and PPIs are commonly prescribed (Larrainzar-Coghen et al., 2016). We need to verify the conditions of all patients, irrespective of their diet, in order to get a reasonable statistic.

Critically ill patients commonly have impaired upper GI function with poor motility and ileus. Such patients are fed with residue-free predigested, or "elemental" form and delivered beyond the stomach into the jejunum. This modification of the normal diet may promote CDI (O'Keefe, 2009). This is because jejunal elemental diets suppress bacteriostatic gastric and pancreaticobiliary secretions. Together they promote colonization of the small intestine with colonic microbiota, leading to small bowel bacterial overgrowth. Secondly, elemental diets are a perfect culture medium for *C. difficile* organisms. Thirdly, elemental diets do not contain complex carbohydrate residues, such as fiber, that escape digestion in the small intestine and enter the colon to provide a fermentable food source for the colonic microbiota. All these factors together should indeed cause tube feed as a risk factor for *C. difficile*, as opposed to our observation.

Abdominal pain and cramping are a well-known symptom for *C. difficile*. This was also reported in a case study where a 76-year-old CDI female patient with profuse abdominal pain but without diarrhea (Singla & Pash, 2020). Similarly, another case study reported abdominal distention and pain in a 66-year-old man in an extended care facility who also had severe constipation (Cowan & Kutty, 2018). Although abdominal pain is considered as a typical clinical symptom of *C. difficile*, we did not enough evidence to conclude that there a significant proportion of the patients in our study exhibited abdominal pain. We need to further investigate this lack of evidence.

A key symptom of *Clostridium difficile* is diarrhea, which is especially seen in elderly hospitalized patients with debilitating underlying conditions who have received antimicrobial agents (Bouza et al., 2005; Gerding et al., 1986; McFarland et al., 1991). This became almost a standard after the 1974 report of Tedesco et al., who first described the importance of nosocomial outbreaks of *C. difficile*-associated diarrhea (Tedesco et al., 1974). Similar findings were observed from studies on extended-care facilities related to outbreaks of diarrhea caused by *C. difficile* (Bender et al., 1986). It is thus not surprising that we see a very significant large number of patients exhibiting diarrhea or loose stool in our study.

According to the literature, a severe CDI could lead to the clinical symptom of fever or temperature above 38.3 ^{o}C (Kelly & LaMont, 2008). In other studies, it was reported that *Clostridium difficile* patients had fever with the following percentages: hospital-acquired patients – 21%; long-term care facility acquired – 24%; and community-acquired – 12.8 % (Garg et al., 2013). Also, high-dose antibiotics tend to significantly lower the fever rates from 8.2 % to 1.9 % in another related study (Ouwehand et al., 2014), suggesting fever is associated with CDI. Although the proportions of the patients who had a fever in our study were not significantly different from those who did not, nevertheless, our quantification revealed that about 34 % of the patients exhibited a temperature above 38 ^{o}C. Thus, our observations are very close to the report on patients with CDAD by McFarland et al., who reported that 30 % of the studied patients also exhibited high fever (McFarland et al., 1999).

The last symptom that we looked at was whether the patients exhibited elevated white blood cell counts (WBC >15 000/µL), which is a prognostic marker in patients with CDI. Although it is not sufficient for the diagnosis of CDI, several reports support the increase in WBC counts in *C. difficile* patients (Wanahita et al., 2003). In fact, WBC counts greater than 15000 / µL is now considered as a criterion for defining severe CDI according to the Infectious Diseases Society of

America / Society for Healthcare Epidemiology of America (IDSA/SHEA) (Bosch et al., 2021; L. C. McDonald et al., 2018; Miller et al., 2013). However, the value of the WBC count in predicting CDI prior to laboratory testing is still not certain. Nevertheless, higher WBC counts can be significantly associated with a positive *Clostridium difficile* toxicity (Potasman & Grupper, 2013). Other publications suggest a sudden WBC increase coinciding with the onset of symptoms suggestive of *C. difficile* (Bulusu et al., 2000). Our results show that three-fourths of the patients had an elevated WBC count. Additionally, the proportion of patients with elevated WBC was significantly higher than those of CDI patients who did not. Our results thus match with published reports that support high WBC count could be a prognostic marker for *C. difficile*.

4.1 Limitations

This study had some limitations. First, the data is collected from only one hospital. Hospitals serve a geographical area within reach of the patients. All geographical areas do not have similar demographic distributions. Hence the patient data may be biased for some of the risk factors.

5.0 Conclusions

The number of CDI patients used antibiotics in greater numbers, whereas significantly fewer infected patients used tube feed. This probably implies that antibiotics could be a risk factor but results on tube feed are inconclusive. The proportion of patients using Proton Pump Inhibitors was similar between users and non-users. There is no difference in the proportion of toxin Positive patients that were on antibiotics, PPIs, and tube feed with those users that were toxin negative. There is no difference in the proportion of patients who had CT <25 and were on medications/therapy (antibiotics, PPIs, and tube feed) and with those who had CT >25.

The proportion of elderly patients, as well as the proportion of White patients, are significantly greater than the younger ones and non-White population, respectively, suggesting *Clostridioides difficile* disproportionately affects the elderly and the White race. *Clostridioides difficile* occurs equally among males and females, and the demographic proportions for Age (>60 vs. <60), gender, and race (White vs. non-White) are similar between Toxin positive and Toxin negatives. There is a statistically significant difference in the proportion of whites who exhibit a CT value greater than 25 than the proportion of whites who exhibit a CT value less than 25, but these are similar for the different age groups and gender.

The significantly greater proportion of *C. difficile*-infected patients exhibited diarrhea / loose stool and high WBC count as diagnostic clinical symptoms. About 30% the patients reported fever that matched with the numbers in reported studies. However, the patients had either abdominal pain or no pain with equal proportions.

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