Clostridioides difficile: The Clinical Effectiveness of Fidaxomicin in Immunocompromised Hosts and the Impact of Resistance Patterns on Clinical Outcomes

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Abstract

Background: *Clostridioides difficile* infection (CDI), a diarrheal illness, is a leading cause of morbidity and mortality worldwide. The risk of developing *C.difficile* infection and recurrence is multifactorial. Patient factors like immunocompromising conditions confer an increased risk of both infection and recurrence. In addition, *C.difficile* strain factors contribute to an added risk of recurrence in epidemic and outbreak settings. In this study, we sought to (1) examine the clinical effectiveness of fidaxomicin compared to vancomycin in treating CDI in patients with immunocompromising conditions and (2) explore the relationship between *C.difficile* antimicrobial resistance and clinical outcomes in both immunocompetent and immunocompromised patients.

Method: This is a single center retrospective study evaluating patients with CDI between 2011-2021. The first part of the thesis included patients with immunocompromising conditions who were treated with oral fidaxomicin or vancomycin. The primary outcome was a composite of failure to achieve clinical cure, relapse at 30 days or CDI-related death. A multivariable cause-specific Cox proportional hazards model was used to test the relationship between treatment and the composite outcome, adjusting for confounders and treating death from other causes as a competing risk. The second part of the thesis describes the antimicrobial resistance patterns of *C.difficile* isolates collected from both immunocompetent and immunocompromised patients during the study period. Isolates were grouped based on resistance. A logistic regression model was used to evaluate the relationship between antecedent antibiotics in the 30 days prior to CDI and resistance among isolates. In addition, an exploratory analysis using a cause-specific Cox

proportional hazards model evaluated the association between resistance and the aforementioned composite outcome.

Results: 238 immunocompromised patients who were treated with either oral fidaxomicin (n =38) or vancomycin (n =200) were included in the first study. There were a total of 53 outcomes, 5 in the fidaxomicin arm and 48 in the vancomycin arm. After adjustment, fidaxomicin significantly decreased the risk of the composite outcome (HR = 0.35, 95% CI 0.12-0.99) and was not significantly associated with other causes of death (HR= 3.0, 95% CI 0.8-11.1). In the second part of the analysis, a total of 510 isolates were analyzed, resistance was noted in 339 (66.5%) of the isolates. Exposure to fluoroquinolones was associated with 2.4 increased odds of developing resistance compared to other antibiotic class exposure (95% CI 1.4-4.4). There were 70 (28.8%) patients in the resistance group and 28 (21.8%) patients in the no resistance group who developed the composite outcome. There was no significant difference in the development of the composite outcome between the two groups (HR = 1.2, 95% CI 0.8-1.9).

Conclusion: The findings of this study suggest that fidaxomicin reduces poor outcomes associated with CDI in immunocompromised patients. With respect to *C.difficile* resistance, fluoroquinolone exposure was significantly associated with isolating a resistant strain, but we did not find significant differences in clinical outcomes based on the presence of antimicrobial resistance.

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Department mentor: Jennifer Chow, MD, MS

Statistical mentor: Angie Mae Rodday, PhD, MS

Program Mentor: David Kent, MD

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List of Abbreviations

CDC	Centers for Disease Control and Prevention
C. difficile	*Clostridioides difficile*
CDI	*C.difficile* infection
HSCT	Hematopoietic stem cell transplant
IDSA	Infectious Disease Society of America
PCR	Polymerase Chain Reaction
SCr	Serum creatinine
SOT	Solid organ transplant
WBC	White Blood Cell

Chapter 1: Introduction

Clostridioides difficile (*C. difficile*) is a gram-positive, spore forming anaerobic bacillus that is the leading cause of nosocomial diarrhea worldwide. The estimated annual incidence of *C.difficile* infection (CDI) in the United States is 450,000 with an estimated 100,000 recurrences and 29,000 deaths (1). This infection negatively impacts patients' quality of life and places a massive burden on the healthcare system. The estimated average of CDI attributable cost per case was $21,448 with a total annual CDI-attributable cost of $6.3 billion in the years 2005-2015 (2).

The clinical spectrum of CDI ranges from asymptomatic carriage to fulminant life-threatening colitis. CDI typically develops following the disruption of the intestinal microbiome which can lead to colonization of the intestinal tract by toxigenic strains of *C. difficile*. These strains release exotoxins leading to colonic mucosal injury by inducing a severe inflammatory response and ultimately host cell death (3).

1.1 Risk Factors for CDI

Antimicrobial exposure is the most important risk factor for acquiring CDI. The number of antecedent antimicrobials, duration, and class are associated with cumulative risk of CDI (4). Antimicrobials can lead to disruption of the normal intestinal microbiome leading to intestinal colonization and infection by *C. difficile.* Similar disruptions can occur with changes in the normal stomach acidity among patients taking gastric acid suppressive agents such as proton pump inhibitors and histamine antagonists (5).

Additionally, patient factors can contribute to increased risk of CDI such as older age and prior hospitalization (6). Further, immunocompromising conditions, such as the

receipt of solid organ transplant (SOT), hematopoietic stem cell transplant (HSCT) or chemotherapy, confer added risk of CDI compared to other hospitalized patients. A meta-analysis assessing studies published between 1991-2014 estimated the prevalence of CDI in SOT recipients to be 7% [95% CI, (5.6-9.5%)] compared to <1% in the general population (7). Similarly, the prevalence of CDI in HSCT recipients ranged between 5-9% which was significantly higher than immunocompetent hospitalized patients (8).

1.2 CDI Outcomes

C.difficile can cause recurrent infection in up to 25% of patients who experience initial response to treatment. With each recurrence, there is an increased risk of complications. Risk factors for recurrence are similar to those for an initial episode. In addition, the concurrent administration of non-CDI antimicrobials during the initial CDI episode significantly increases the risk of recurrence (9).

Another factor affecting *C.difficile* clinical presentation and outcome is infection with different *C.difficile* strains. Between 2000 and 2010, the rates of infection and *C.difficile* recurrence were increasing in North America and Europe. This change was attributed to the emergence of a hypervirulent strain of *C.difficile* known as NAP1/B1/027 (10, 11). Additionally, higher rates of treatment failure were observed following the use of vancomycin and metronidazole during the same period, which was likely related to outbreaks with this strain (12, 13). Other strains, including ribotypes 078 and 001, have been implicated in severe disease in outbreaks (14, 15).

1.3 CDI Treatment

Given the complexity of CDI infection and risk of recurrence, the goal of CDI treatment is not only to resolve the acute illness but also minimize recurrences. There are currently three agents that are commonly used for the treatment of CDI including fidaxomicin, vancomycin and metronidazole. Previously published guidelines by the Infectious Disease Society of America (IDSA) in 2017 recommended the use of oral vancomycin or fidaxomicin for an initial CDI episode with metronidazole as an alternative if access to either was limited (3). An update on the guidelines published in 2021 recommended the use of fidaxomicin over vancomycin for both initial and recurrent episodes (16). These recommendations were made based on industry sponsored randomized control trials that showed high rates of sustained clinical cure in the fidaxomicin group versus vancomycin (17-20). However, the populations in the studies were mostly immunocompetent patients with limited history of immunocompromising conditions.

There have been a few studies looking at the efficacy of fidaxomicin for the treatment of CDI in populations with immunocompromising conditions. A *post hoc* analysis of two double-blinded randomized control trials showed that fidaxomicin use in cancer patients had significantly higher rates of sustained clinical cure and fewer recurrences compared to vancomycin (21). Another observational retrospective study comparing vancomycin to fidaxomicin in HSCT found no differences in initial resolution of diarrhea or global cure without recurrence (22).

Therefore, in light of the limited evidence, the purpose of the first part of this study was to evaluate the clinical effectiveness of fidaxomicin compared to vancomycin

in patients with immunocompromising conditions. The primary outcome evaluated was a composite outcome of failure to achieve clinical cure, relapse of CDI within 30 days from treatment completion or death related to CDI. The components of the composite outcome were investigated separately, in addition to two secondary outcomes of relapse 90 days and total relapse from treatment completion. For the second part of this study, we assessed antecedent antibiotic exposure and its relationship to *C.difficile* resistance and ribotypes among both immunocompetent and immunocompromised patients. An exploratory analysis was conducted to examine the impact of *C.difficile* resistance patterns on clinical outcomes including the aforementioned composite outcome and its components. The findings from both parts of this study could advance the knowledge of CDI treatment in immunocompromised populations and address the effect of *C.difficile* resistance on clinical outcome.

1.4 Student Contribution

This chapter's literature review was performed exclusively by Majd Alsoubani.

Chapter 2

The Comparative Effectiveness of Fidaxomicin compared to Vancomycin in Populations with Immunocompromising Conditions [1]

[1] Alsoubani M, Chow JK, Rodday AM, Kent D, Snydman DR. To be submitted to American Journal of Transplantation

2.1 Introduction

Clostridioides difficile (*C. difficile*) is a leading cause of health care associated diarrhea worldwide and patients with immunocompromising conditions are at increased risk of *C.difficile* infection (CDI) compared to other hospitalized patients (23). The prevalence of CDI in solid organ transplant (SOT) recipients is up to 7-fold higher compared with the general population and it is particularly higher in the first three months post-transplant (7, 24). Among hematopoietic stem cell transplant (HSCT) recipients, the prevalence of CDI ranges between 5 and 9% (8), with a median time to diagnosis of 6-33 days post-transplant (25). The increased prevalence in this population is likely related to hospitalization, increased exposure to broad spectrum antimicrobials and intense immunosuppression in the immediate post-transplant period (25, 26). Late onset CDI, occurring months to years post-transplant is typically associated with exposure to antimicrobials or episodes of rejection requiring intensification of immunosuppression (26).

The presence of CDI in SOT recipients is associated with worse outcomes, including increased in-hospital mortality, organ failure or rejection and longer hospital stays compared to other SOT recipients (27, 28). Moreover, in HSCT recipients, there is a strong correlation between CDI and subsequent acute graft versus host disease (GVHD) (25). Immunocompromised status confers more than twice the risk of recurrence compared to the general population (29). The risk of recurrence of CDI in patients after HSCT ranges between 20-40% and upwards of 40% in patients with SOT (30, 31). Risk factors for recurrence include recent hospitalization, antimicrobial use prior to CDI, older age and renal failure (32).

An update of CDI treatment guidelines was published in 2021 recommending the use of fidaxomicin over vancomycin for both initial and recurrent episodes of CDI (16). The recommendations were made based on the results of randomized controlled trials including mostly immunocompetent patients without history of transplantation or use of immunosuppressants (17-20). On the other hand, evidence on the efficacy of fidaxomicin in populations with immunocompromising conditions is sparse. A *post hoc* analysis of two double-blinded randomized control trials including 183 patients with a cancer diagnosis, showed that fidaxomicin use in cancer patients had significantly higher rates of sustained clinical cure and fewer recurrence (33). However, the majority,150 patients, were not on active chemotherapy treatment. Another observational retrospective study comparing vancomycin to fidaxomicin in 96 recipients of HSCT found no differences in initial or global cure of CDI (22).

There is limited data on fidaxomicin effectiveness in patients with immunocompromising conditions. Therefore, the purpose of this study is to compare the clinical outcomes of CDI following treatment with fidaxomicin compared to vancomycin in patients with immunocompromising conditions.

2.2 Methods

2.2.1 Study Setting

This was a retrospective study conducted at Tufts Medical Center, a tertiary care academic hospital in Boston, MA. The study examined patients with immunocompromising conditions who were diagnosed with CDI from January 1, 2011 to December, 31, 2021.

Patients were classified as having an immunocompromising condition if they met at least one of the following criteria at the time of CDI diagnosis: (1) having a solid or hematologic organ transplant at any time prior to being diagnosed with CDI, (2) undergoing active chemotherapy for leukemia, lymphoma, or solid tumors, or (3) being on immunomodulator agents (Appendix Table 5.1). Patients less than 18 years of age, those who did not receive any treatment for CDI, and those treated with metronidazole only or fecal microbiota transplant were excluded. Demographic characteristics of the patients who were excluded were outlined in Appendix 5.1.1.

The Tufts Medical Center Institutional Review Board approved this study (SUDY00001199).

2.2.2 Definitions

C.difficile infection (CDI) was defined as a diarrheal illness with a positive stool test for *C.difficile* which required the initiation of treatment by the treating provider. A test was considered positive if both glutamate dehydrogenase antigen and toxin assays were positive or nucleic acid amplification test was positive (3).

Index CDI was defined as the first episode of CDI with a documented test in the medical record after the diagnosis of the immunocompromising condition. Patients with previous episodes of CDI as reported in the clinical notes were defined as having history of CDI.

Failure to achieve clinical cure was defined as the presence of persistent diarrhea despite medical treatment as determined by the treating physician within 72 hours of treatment initiation.

Relapse at 30 days was defined as recurrence of CDI or the need to restart CDI treatment within 30 days (+7 days) of stopping therapy for the index CDI case, as determined by the treating physician.

Relapse at 90 days was defined as recurrence of CDI or the need to restart CDI treatment between 30 and 90 days (+7 days) of stopping therapy for the index CDI case, as determined by the treating physician, excluding relapses that occurred before 30 days.

Total relapse was defined as recurrence of CDI or the need to restart CDI treatment within 90 days (+7 days) of stopping therapy for the index CDI case, as determined by the treating physician.

Death related to CDI was defined as any death that was attributed to CDI within 30 days of initial diagnosis. This included death from fulminant colitis or septic shock.

Death from other cause, a competing risk to the primary outcome, was defined as any death that was not associated with CDI within 30 days of CDI diagnosis. This included cardiac, pulmonary or cancer related deaths.

Severe CDI was defined based on IDSA criteria; leukocytosis with white cell count (WBC) of $\geq$ 15,000 cells/ml or serum creatinine (SCr) >1.5 mg/dl (3).

Hospital acquired CDI was defined as infection diagnosed within 48 hours following admission to the hospital.

Health care associated CDI was defined as exposure to a health care facility within 30 days prior to diagnosis.

2.2.3 Data Collection

CDI cases in immunocompromised patients who satisfied our inclusion criteria were abstracted from the microbiology and hospital databases. All demographic and clinical data including age, sex, race, ethnicity, comorbid conditions, history of prior CDI, the use of gastric acid suppression, toxin test positivity, location prior to and at the time of CDI diagnosis and intensive care unit (ICU) admission were collected retrospectively from the electronic medical record. The Charlson comorbidity Index (CCI) was used to stratify patients based on their comorbidities (34). Antecedent antibiotic exposure was limited to 30 days prior to index case diagnosis. Laboratory data, including white blood cell count (WBC) and serum creatinine (SCr), were collected at the time of CDI diagnosis. If there were multiple values on the same day, the one closest to the time of CDI testing was recorded. Study data was collected using REDCap (Research Electronic Data Capture) hosted by Tufts Medical Center. REDCap is a secure, web-based application designed to support data capture for research studies (35).

Cases where death occurred in the first 30 days following the diagnosis of CDI were reviewed independently by two physicians (MA, CT) who were blinded to treatment. Death was adjudicated as death attributed or contributed to CDI or death from another cause. There were no discrepancies in adjudication between the two reviewers.

2.2.4 Primary Study Exposure and Clinical Outcomes

The main exposure variable of interest was CDI directed antibiotics. At Tufts Medical Center, during the study period, fidaxomicin was the recommended regimen for patients with increased risk of recurrence including those who had chronic kidney disease, a history of CDI, were older and were immunocompromised. Vancomycin was

recommended for all other patients. Physicians were allowed to choose either treatment regimen. Patients were classified as being treated with one antibiotic versus the other if they received at least 72 hours of the agent. An additional sensitivity analysis was done to evaluate the outcome based on the first antibiotic received for treatment.

The primary study outcome was a composite outcome of failure to achieve clinical cure within 72 hours of treatment initiation, relapse within 30 days following completion of initial CDI treatment and having died due to CDI. Each component of the composite outcome was analyzed separately in addition to the secondary outcomes of relapse at 90 days and total relapse following completion of initial CDI treatment.

For the composite outcome, patients were considered lost to follow up, if there was no documentation of clinical status in the medical record by 30 days following completion of treatment. The date of last known follow up was determined by the date of discharge from the hospital or the last known outpatient clinic visit.

2.2.5 Statistical Analysis

We performed multiple imputation to estimate missing values for laboratory and clinical data using a logistic regression model (36-38). Ten data sets were imputed, and pooled estimates were used for the analyses. Missing data was assumed to be missing at random, the demographic and clinical characteristics among patients with and without loss of follow up is shown in Appendix Table 5.5.

Patient characteristics by treatment group were presented as counts and percentages for categorical variables and medians with interquartile range for continuous variables if they were skewed; we tested for differences using Mann-Whitney or chi-

square test. For the primary composite outcome, time 0 was date of CDI diagnosis; patients without an event by 30 days of treatment completion were censored, and patients were censored earlier based on time of last known follow up. The primary analysis was a time to event analysis using cause specific Cox proportional hazards comparing the rate of the composite outcome following treatment with fidaxomicin compared to vancomycin. A cause specific Cox proportional hazard model was used to account for competing risk of death from other causes. Univariate and multivariate proportional hazard models were evaluated. Candidate variables that may confound the relationship between treatment and the primary outcome were included in the final multivariate model based on clinical knowledge and the collapsibility approach (i.e., change in beta coefficient by 20%); we limited the number of confounders to 5 based on the number of study outcomes. The proportional hazards assumptions were checked using graphical assessment of Schoenfeld residuals and Log(-log) plots (39).

The components of the composite outcome including failure to achieve clinical cure, relapse at 30 days and CDI related death, as well as the secondary outcomes of relapse at 90 days and total relapse, were examined individually using cause specific Cox proportional hazards model. Unlike the primary analysis of the composite outcome, time 0 was date of treatment completion for relapse by 30 or 90 days; patients without outcome were censored at 30 or 90 days, respectively or at the date of last known follow up. respectively.

There were 10 patients in the vancomycin group who were continued on CDI treatment for more than 30 days compared to none in the fidaxomicin group creating a potential for immortal time bias when evaluating 30 day relapse in the composite

outcome. Immortal time bias occurs when participants have an interval during which the outcome event cannot occur (40). We conducted a sensitivity analysis excluding these patients to assess the impact of immortal time bias on the outcome.

In the primary analysis, treatment was based on CDI therapy administered for at least 72 hours. We conducted a sensitivity analysis to evaluate the intention to treat effect by assessing the relationship between outcome and first dose of CDI therapy received.

All statistical analyses were completed using either R Studio software version 4.1.2 (R Core Team, Vienna, Austria) or SPSS version 28. A p-value of <0.05 was considered statistically significant unless otherwise indicated.

2.3 Results

2.3.1 Patient Characteristics

A total of 844 patients were diagnosed with CDI during the study period January 1, 2011 to December, 31, 2021 (Appendix 5.1.3). Among the 298 patients with immunocompromising conditions, 43 patients were excluded for receiving metronidazole alone for treatment and 17 patients for not receiving any CDI-directed treatment, resulting in a final sample size of 238 (Figure 2.1). The median duration of treatment with vancomycin was 13 days (QI 10,15) and 12 days (QI, 11,15) for fidaxomicin.

Patients who received vancomycin were significantly more likely to be male (51.5% vs 26.2%, p=0.005) and to have had community acquired infection (31.1% vs 9.5%, p=0.03) compared to the fidaxomicin treatment group (Table 2.1). There were no other significant differences in demographic and clinical characteristics by treatment.

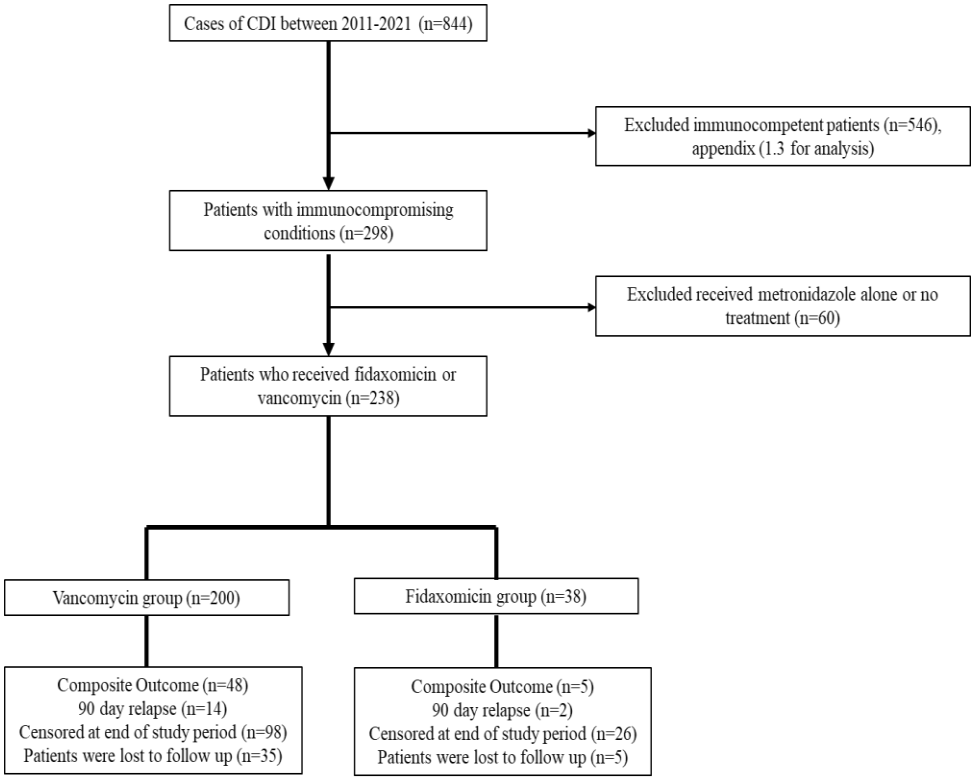

Figure 2.1 Flow diagram of study participant selection and outcomes

2.3.2 Clinical Outcomes

A total of 53 (22.3%) patients developed the composite outcome. Of those who developed the composite outcome, 22 (9.4%) patients failed to achieve clinical cure and 26 (12.3%) relapsed within 30 days of completing treatment. There was a total of 17 deaths in the cohort during the follow up period, with 5 deaths adjudicated as CDI related while the rest were considered to be CDI unrelated deaths (Appendix 5.1.4). The composite outcome occurred in 48 (24%) of patients in the vancomycin group compared to 5 (13.2%) of patients in the fidaxomicin group. The distribution of the primary and secondary outcomes by treatment group is displayed in Table 2.2. The median censored time for those who were lost to follow up was 20 days (QI 11.5, 34.0).

Table 2.1 Demographic and clinical characteristics of patients by treatment [a]			
	Fidaxomicin (n=38)	Vancomycin (n=200)	p-value
Age, Median (Q1,Q3) [b]	62.9 (56.7, 74.1)	62.5 (53.4,70.7)	0.36
Male, n (%)	10 (26.3)	103 (51.5)	0.005
White, n (%)	31 (80.5)	148 (74.1)	0.42
Hispanic, n (%)	3 (8.4)	11 (5.3)	0.49
Type of immunosuppression, n (%)			
SOT	19 (50)	57 (28.5)	0.29
BMT	5 (13.2)	19 (9.5)	
Leukemia/lymphoma	5 (13.2)	43 (21.5)	
Solid tumor	4 (10.5)	40 (20.0)	
Immunomodulator	5 (13.2)	41 (20.5)	
CCI, Median (Q1, Q3) [b]	5 (3,7)	5 (3,8)	0.50
Dialysis, n (%)	7 (18.4)	16 (8.0)	0.07
CDI in past 6 months, n (%)	2 (5.3)	2 (1.2)	0.09
Gastric acid suppression, n (%)	30 (78.9)	111 (55.4)	0.007
Location prior to diagnosis, n (%)			
Hospital acquired	20 (53.4)	82 (41.1)	0.03
Health care associated	14 (37.1)	56 (27.9)	
Community acquired	4 (9.5)	62 (31.1)	
Inpatient diagnosis, n (%)	36 (94.7)	167 (83.5)	0.07
Toxin test, n (%)	20 (51.3)	116 (58.2)	0.45
WBC count, Median (Q1,Q3) [b]	6900.0 (4950, 12200)	7600.0 (3900, 12775)	0.96
Severe CDI, n (%)	16 (42.4)	75 (37.7)	0.59
ICU stay, n (%)	8 (19.7)	37 (18.4)	0.84
Antecedent antibiotic use, n (%)	30 (77.9)	146 (73.0)	0.55
Antibiotics during treatment, n (%)	18 (47.4)	105 (52.5)	0.60
Number of antecedent antibiotics, Median (Q1,Q3) [b]	2 (1,3)	1 (0,2)	0.07

Abbreviations: Q1 =quartile 1, Q3 = quartile 3, CCI =Charlson comorbidity index, WBC = white blood cell count, CDI = *Clostridioides difficile* infection, ICU = intensive care unit

[a] Chi-square was used for all testing unless otherwise specified

[b] Mann-U-Whitney

Table 2.2 Differences in the primary and secondary outcomes according to treatment group

	Fidaxomicin (n = 38)	Vancomycin (n = 200)	Total (n = 238)
Primary outcome			
Composite outcome n, (%) [a]	5 (13.2)	48 (24.0)	53 (22.3)
Secondary outcomes			
Failure to achieve clinical cure n, (%)	3 (7.9)	19 (9.5)	22 (9.2)
30 day relapse n, (%) [b]	1 (2.9)	25 (14.1)	26 (12.3)
90 day relapse n, (%) [c]	2 (6.1)	14 (8.0)	16 (8.6)
Total relapse n, (%)	3 (8.8)	39 (22.0)	42 (19.9)
Death related to CDI n, (%)	1 (2.9)	4 (2.2)	5 (2.3)

[a] Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI
[b] n after exclusion of patients who failed to achieve clinical cure or died
[c] n after exclusion failed to achieve clinical cure, died or had 30 day relapse

Table 2.3 displays the unadjusted and adjusted hazard ratios associated with fidaxomicin treatment for the composite outcome and competing risk of death from other cause. In the multivariate model, following adjustment for confounding variables, fidaxomicin was associated with 65% reduction in the hazard of developing the composite outcome compared with vancomycin (HR = 0.35, 95% CI 0.12-0.99). There was no significant relationship between fidaxomicin and other causes of death after adjustment. A sensitivity analysis based on the first dose of CDI directed therapy received showed similar results (Appendix 5.1.5).

Table 2.3 Summary of unadjusted and adjusted cause specific proportional hazard model in immunocompromised patients				
	Composite outcome*		Other causes of death	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Unadjusted model				
Fidaxomicin	0.58 (0.23-1.5)	0.25	3.7 (1.2-12.0)	0.03
Adjusted model**				
Fidaxomicin	0.35 (0.12-0.99)	0.049	3.0 (0.8-11.1)	0.10
*Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI **Adjusted for sex, number of antecedent antibiotics, antibiotics during treatment, severity and type of immunosuppression				

The relationship between treatment and the individual components of the composite outcome and the secondary outcomes were explored in Table 2.4. The hazard of total relapse was significantly lower in the fidaxomicin compared to vancomycin in the multivariable adjusted model (OR = 0.3, 95% CI 0.1-0.9).

Table 2.4 Summary of unadjusted and adjusted proportional hazard for fidaxomicin and the secondary outcomes in immunocompromised patients*				
	Unadjusted hazard ratio (95% CI)	p-value	Adjusted hazard ratio (95% CI)	p-value
Failure to achieve clinical cure	0.8 (0.2-2.7)	0.79	0.6 (0.1-2.9)	0.65
30 day relapse	0.2 (0.03-1.6)	0.10	02 (0.02-1.2)	0.07
90 day relapse	0.7 (0.2-2.9)	0.57	0.5 (0.1-2.4)	0.50
Total relapse	0.4 (0.12-1.2)	0.09	0.3 (0.1-0.9)	0.03
CDI related death	1.7 (0.2-16.0)	0.66	0.7 (0.1-9.8)	0.82
*Adjusted for sex, number of antecedent antibiotics, antibiotics during treatment, severity and type of immunosuppression				

We conducted two different sensitivity analyses the first after excluding patients who received longer treatment with vancomycin and the second was based on the first dose of treatment received. The hazard ratio was similar to the main analysis (Appendix 5.1.5 and 5.1.6).

2.4 Discussion

We evaluated the clinical effectiveness of fidaxomicin compared to vancomycin in preventing CDI treatment failure in patients with immunocompromising conditions. Our findings suggest that fidaxomicin offers protection against CDI treatment failure compared with vancomycin.

Vancomycin was largely prescribed for initial and recurrent episodes of CDI which was consistent with the IDSA CDI guideline recommendations at the time the study was conducted (3). It did not reflect the 2021 IDSA and European Society of Clinical Microbiology and Infectious Diseases guidelines updates recommending fidaxomicin as the first line of treatment (16, 41). Although, our institutional guidelines recommended fidaxomicin use in a larger proportion of patients than what was seen in this study, prescription of fidaxomicin can be limited by cost and insurance coverage.

In our study, patients who received fidaxomicin had a 65% reduction in the hazard of treatment failure. These results are similar to a *post-hoc* analysis of oncology patients who participated in fidaxomicin or vancomycin treatment randomized controlled trials where the odds of CDI recurrence in the fidaxomicin group was significantly lower (OR = 0.37) than the vancomycin group (22). Furthermore, in a prospective study comparing immunocompromised patients to other hospitalized patients, the use of

vancomycin was found to be a significant predictor of CDI recurrence (29). There was a signal towards increased hazard of other cause of death in the fidaxomicin group, however upon investigating the cause of death in this group, all patients died from chronic longstanding conditions that were present prior to CDI diagnosis.

The total relapse rate (30 and 90 days) was significantly lower in the fidaxomicin group than the vancomycin group in the present study. The overall total rate of relapse reported in this study was 19.9% which is slightly lower than previously reported relapse rates of 25-40% in other studies of immunocompromised patient populations (30, 32, 42).

Each CDI recurrence poses an increased risk of clinical complications and adverse outcomes. The risk of sepsis and colectomy increases with each recurrent CDI episode (43). Other complications specific to transplant recipients include rejection of the transplanted organ and increased risk of GVHD (27) (44) (45). The negative impact of CDI recurrence extends beyond clinical complications. Health related quality of life scores were lower in patients with CDI who reported greater impairment in work and other activities compared to patients without CDI (46, 47). Furthermore, CDI recurrence places increased burden on healthcare resource utilization and direct medical costs (48). Thus, identifying agents that decrease the risk of recurrence among patients with CDI remains sorely needed.

There are several limitations in this study. This was a retrospective single center study design which led to missing data in baseline variables and some patients who were censored based on when they were lost to follow up. We used a Cox proportional hazards model to control for those patients with missing outcomes by censoring at the time of last known follow up. Multiple imputation was used for baseline variables. As an

observational study, treatment decisions were determined by the treating physician, so there may be confounding by indication, though we adjusted for several variables to reduce this bias, the possibility of residual confounding remains, both because there may be unobserved confounders and because we had limited power to adjust for observed confounders. Patients were categorized to treatment groups based on the agent used for 72 hours or more, but a small number of patients received a different agent for the first dose. However, this was addressed by a sensitivity analysis exploring an intention-to-treat approach which showed similar results to the primary analysis. Since treatment duration varied slightly in the vancomycin group compared to fidaxomicin group, this created the potential for immortal time bias which was addressed by a sensitivity analysis excluding these patients with similar results compared to the main analysis. Strengths of this study include the utilization of cause specific Cox proportional hazard model to account for the competing risk of death from other causes. In addition, the study has a larger cohort of immunocompromised patients than previously studied, although the power of the study was limited.

2.5 Student contribution

Majd Alsoubani performed the literature review, statistical analysis and wrote all aspects of the chapter.

Chapter 3

Clostridioides difficile resistance: the impact of antecedent antibiotic exposure and the relationship with clinical outcomes [1]

[1] Alsoubani M, Chow JK, Rodday AM, Kent D, Snydman DR. To be submitted to Anaerobe journal

3.1 Introduction

Clostridioides difficile (*C. difficile*) is an important pathogen causing health-care associated diarrhea worldwide. It is currently listed by the Center for Disease Control and Prevention (CDC) as a pathogen associated with urgent threat due to increased antimicrobial resistance (49). *C.difficile* is a gram-positive, spore forming anaerobic bacillus that is transmitted in humans via endospores, which are highly resistant to a wide range of disinfectants (50). The endospores can germinate into the vegetative state in the host leading to intestinal colonization, toxin production and subsequently clinical manifestations of CDI. Most *C.difficile* strains are capable of producing two toxins TcdA (Toxin A) and TcdB (Toxin B). These toxins disrupt the cytoskeletal structure of the intestinal cells leading to cell death which ultimately contributes to the development of fulminant disease (51).

Previous antibiotic use is the main risk factor for developing CDI by changing the composition of the normal intestinal microbiome. Antimicrobial resistance patterns detected in *C.difficile* can enhance pathogenicity when the rest of the gut microbiome is disrupted following exposure to antimicrobials and can augment the risk of infection (52). This is reflected by an increased risk of CDI following exposure to fluroquinolones, clindamycin and cephalosporin antibiotics due to the higher rates of resistance in *C.difficile* strains (53-55).

In addition to resistance testing, the identification and classification of *C.difficile* strains have been performed by using different molecular techniques. Polymerase chain reaction (PCR) ribotyping has been shown to be a reliable and reproducible method to identify different *C.difficile* strains (56, 57). The epidemiology of CDI became more

relevant following the identification and emergence of the epidemic ribotype 027 in the early 2000s which contributed to a number of health care associated outbreaks and increased CDI severity and mortality (58). Access to ribotyping data has been used in CDI control programs in implementing directed infection control measures leading to changes in the prevalence of different epidemic strains (59). Surveillance programs in the US and Europe have shown a marked decline in ribotype 027 accompanied by the emergence of other ribotypes including 106 and 107 during more recent years (60, 61).

In this observational study, we sought to examine the patterns of CDI resistance and ribotypes as well as their impact on CDI related clinical outcomes. We conducted several exploratory analyses to understand the following relationships: (1) antecedent antibiotic exposure and the isolation of CDI resistance, (2) CDI resistance and clinical outcomes and (3) CDI ribotype and the pattern of CDI resistance.

3.2 Methods

3.2.1 Study Setting

This was a retrospective study conducted at Tufts Medical Center, a 415-bed tertiary academic hospital in Boston, MA. The study included stool samples from patients who were diagnosed with CDI from January 1, 2011 to December, 31, 2020. Patients with multiple samples had only their initial sample included in this study. Samples from patients less than 18 years old were excluded. The Tufts Medical Center Institutional Review Board approved this study (SUDY00001199).

3.2.2 Data collection and procedures

At Tufts Medical Center, we have collected more than 600 *C.difficile* isolates initially collected as part of surveillance for fidaxomicin susceptibility required by the FDA between the years 2011-2016 and then as part of our internal surveillance program (62).

The Special Studies laboratory at Tufts Medical Center routinely obtained stools from patients who were determined to have *C.difficile* infection and cultured them (60, 63). The isolates of *C.difficile* obtained from stool samples were ribotyped by PCR-based fragment analysis at the Seth Walk laboratory (60). Susceptibility testing was performed on all isolates using the Clinical & Laboratory Standards Institute (CLSI) recommended methodology. Antibiotic agents tested included: fidaxomicin, rifaximin, rifampin, tigecycline, vancomycin, imipenem, moxifloxacin, metronidazole, clindamycin and chloramphenicol. Minimal inhibitory concentration (MIC) results were determined by agar dilution and were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) epidemiologic cut off (ECOFF) values or the CLSI breakpoints, when applicable (Appendix Table 5.15) (64, 65). Isolates were considered resistant if the MIC exceeded either EUCAST or CLSI breakpoints.

Patient demographic and clinical data including age, sex, race, ethnicity, comorbid conditions, history of prior CDI, the use of gastric acid suppression, toxin test positivity, location prior to and at the time of CDI diagnosis and intensive care unit (ICU) admission were collected from the hospital medical records during the study period. Antecedent antibiotic exposure was limited to the 30 days prior to isolate collection as

recorded in the medical chart or pharmacy records. The Charlson comorbidity Index (CCI) was used to stratify patients based on their comorbidities (34).

3.2.3 Definitions

C.difficile infection (CDI) was defined as a positive stool test for *C. difficile*. A test was considered positive, if both glutamate dehydrogenase antigen and toxin assays were positive or nucleic acid amplification test was positive.

Multi-drug resistant isolate was defined as the presence of resistance pattern to three or more agents from different categories.

Health care associated CDI was defined as infection diagnosed during the first 48 hours following admission to the hospital or prior health care exposure within 30 days prior to diagnosis.

CDI directed treatment was defined as the receipt of fidaxomicin, vancomycin or metronidazole based on the agent used for at least 72 hours.

Immunocompromised patients were defined as patients having an immunocompromising condition if they met at least one of the following criteria at the time of CDI diagnosis: (1) having a solid or hematologic organ transplant at any time prior to being diagnosed with CDI, (2) undergoing active chemotherapy for leukemia, lymphoma, or solid tumors, or (3) being on immunomodulator agents.

3.2.4 Primary Study Exposures and Outcomes

The primary exposure of interest was antecedent antimicrobial class exposure within 30 days prior to CDI specimen isolation. This time period was selected based on review of the previous literature and the availability of retrospective data. Antimicrobials

were grouped based on class (Appendix Table 5.16). The primary study outcome was isolate antimicrobial resistance following antimicrobial exposure.

Additional secondary analyses were performed examining the presence of CDI resistance in relation to the composite clinical outcome of failure to achieve clinical cure despite treatment, relapse within 30 days following completion of initial CDI treatment and death related to CDI (definitions included in Appendix 5.2.1). Each component of the composite outcome was analyzed separately in addition to the secondary outcome of relapse at 90 days following completion of initial CDI treatment.

3.2.5 Statistical Analysis

Patient's descriptive characteristics were presented as frequencies and medians with interquartile ranges according to the presence of an antibiotic resistance; we tested for between group differences in these factors using chi-square and Mann-Whiney tests respectively. The primary analysis utilized a logistic regression model assessing the relationship between antecedent antibiotic exposure and the emergence of *C.difficile* resistance. The multivariate model was built by first conducting a univariate screen ($p<0.1$) examining the association between antibiotic class and resistance. Antibiotics that were significant in the univariate screen were included in the final multivariate model. Clinical variables were also included in the model based on clinical knowledge and a univariate screen ($p <0.1$) to adjust for potential confounding.

Multiple imputation was performed to retain cases with missing variables in the analysis under the missing at random assumption (36-38). Ten complete data sets were imputed and pooled estimates were used for the analysis. The frequency of missing values per variable is highlighted in Appendix 5.2.2. Only 15 (2.9%) of patients had

missing data on antecedent antibiotic exposure. A comparison of the clinical and demographic characteristics between patients with and without loss of follow up is shown in Appendix Table 5.17. The analysis was done using chi-square or Fisher's exact tests and Mann-U-Whitney for categorical and continuous variables, respectively.

An exploratory analysis evaluating the relationship between resistance and the composite clinical outcome was conducted. We described the frequency of patients with the composite outcome, and its individual components, by resistance. For the composite clinical outcome, time 0 was date of CDI diagnosis; patients without an event by 30 days of treatment completion were censored, and patients were censored earlier based on time of last known follow up. A time to event analysis of the composite outcome by resistance using cause specific Cox proportional hazards accounted for competing risk of death from other causes.

The components of the composite outcome including failure to achieve clinical cure, relapse at 30 days and CDI related death, as well as the secondary outcome of relapse at 90 days, were examined individually using Cox proportional hazards model. Unlike the primary analysis of the composite outcome, time 0 was date of treatment completion for relapse by 30 or 90 days; in addition, for 90 day relapse, censoring occurred at 90 days from treatment completion in the absence of relapse. Univariate and multivariate models were evaluated. Variables included in the model were determined based clinical knowledge and on a univariate screen ($p < 0.1$). The proportional hazards assumptions were checked using graphical assessment of Schoenfeld residuals and Log(-log) plots (39). We also included a descriptive analysis of resistance pattern and clinical outcomes by ribotype.

3.3 Results

Antibiotic susceptibility testing was completed on *C.difficile* isolates from 510 patients of which 498 were ribotyped successfully.

3.3.1 *C.difficile* Resistance Patterns

A total of 339 (66.5%) isolates were resistant to at least one antimicrobial agent. 107 (21%) of the isolates had resistance to two classes of antimicrobials, while 59 (11.4%) isolates were multidrug resistant. Table 3.1 displays resistance by CLSI and/or EUCAST breakpoint recommendations.

Table 3.1 Antimicrobial susceptibility testing for *C.difficile* isolates

Antimicrobial agent	Number of resistant isolates (%)	
	CLSI [1]	EUCAST [1]
Fidaxomicin	NA	NA
Rifaximin	NA	NA
Rifampin	NA	194 (38.0)
Vancomycin	NA	34 (6.7)
Metronidazole	NA	10 (2.0)
Moxifloxacin	144 (28.2)	144 (28.2)
Clindamycin	167 (32.7)	NA
Imipenem	29 (5.7)	NA
Tigecycline	NA	2 (0.4)
Chloramphenicol	NA	NA

[1] NA not applicable

3.3.2 Antimicrobial Exposure and Resistance

Patients who had isolates exhibiting at least one antimicrobial resistance were more likely to be older and have a higher CCI score at the time of diagnosis as compared to those without resistance (Table 3.2). Furthermore, antimicrobial resistance was more

frequently detected in health care associated infections compared to community acquired infections. Appendix 5.2.3 describes patients with multidrug resistant isolates.

Table 3.2 Patient Demographic and Clinical Characteristics of *C.difficile* isolates exhibiting at least one antimicrobial resistance [a]			
	At least one antibiotic resistance detected (n = 341)	No Resistance (n = 169)	p-value
Age (yr), Median (Q1,Q3)[b]	65.9 (54.4, 73.8)	60.7 (51.5, 72.4)	0.048
Male, n (%)	168 (49.3)	98 (58.0)	0.07
White, n (%)	254 (74.5)	128 (75.7)	0.83
Hispanic, n (%)	15 (4.4)	8 (4.7)	0.72
Immunocompromised, n (%)	108 (31.7)	50 (29.6)	0.61
CCI Median, (Q1,Q3)[b]	4 (3,6)	4 (2, 5)	0.02
Dialysis, n (%)	19 (5.7)	12 (7.1)	0.08
History of CDI, n (%)	11 (3.1)	5 (3.0)	0.99
Gastric Acid Suppression, n (%)	206 (60.4)	88 (52.1)	0.07
Health care associated, n (%)	252 (73.8)	108 (64.2)	0.03
Inpatient testing, n (%)	284 (83.4)	131 (77.6)	0.12
Toxin Test, n (%)	198 (57.9)	89 (52.5)	0.25
WBC, Median (Q1,Q3)[b]	11200 (6750, 17000)	9050 (6100, 14500)	0.26
Severe CDI, n (%)	147 (43.0)	60 (35.4)	0.11
ICU, n (%)	84 (24.5)	32 (18.8)	0.16
Any antecedent antimicrobial class exposure, n (%)	265 (77.7)	110 (65.3)	0.003
Two or more antecedent antimicrobial class exposure, n (%)	157 (46.0)	60 (35.5)	0.04
Antimicrobials during treatment, n (%)	188 (55.1)	87 (51.7)	0.46
Number of antecedent antimicrobials, Median (Q1,Q3)[b]	1 (1,3)	1 (0,2)	0.003
a Chi-square test unless otherwise specified. b Mann U Whitney test			

Forty three percent of study patients received two or more antibiotics prior to CDI. Only 20 (23.5%) patients received fluoroquinolone monotherapy while monotherapy with cephalosporins and azithromycin were administered in 50 (25.5%) and 4 (18.2%) patients respectively. Patients with evidence of resistance were more significantly more likely to have received more than one antibiotic prior to CDI as compared to patients without resistance (46.0% vs 35.5%, p=0.04).

Table 3.3 Univariable and multivariable analysis of antecedent antibiotic class and the presence of resistance

	Resistance (n =332)	No resistance (n=163)	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)*	p-value
Aminopenicillins, n (%)	27 (8.1)	14 (8.6)	0.94 (0.5-1.9)	0.86		
Cephalosporins, n (%)	142 (42.8)	54 (33.1)	1.5 (1.0-2.2)	0.04	1.4 (0.9-2.1	0.09
Carbapenems, n (%)	50 (15.1)	22 (13.5)	1.1 (0.7-2.0)	0.64		
Aminoglycosides, n (%)	2 (0.6)	2 (1.2)	0.49 (0.1-3.5)	0.48		
Fluoroquinolones, n (%)	69 (20.8)	16 (9.8)	2.4 (1.3-4.3)	0.003	2.4 (1.4-4.4)	0.003
Piperacillin-tazobactam, n (%)	31 (9.3)	16 (9.8)	0.95 (0.5-1.8)	0.86		
Vancomycin, n (%)	110 (33.1)	46 (28.2)	1.3 (0.8-1.9)	0.27		
Metronidazole, n (%)	29 (8.7)	13 (8.0)	1.1 (0.6-2.2)	0.78		
Azithromycin, n (%)	20 (6.0%)	2 (1.2%)	5.2 (1.2-22.4)	0.03	4.7 (1.1-20.5)	0.04
Clindamycin, n (%)	8 (2.4)	3 (1.8)	1.3 (0.4-5.0)	0.69		
Other antibiotics, n (%)	38 (11.4)	16 (9.8)	1.2 (0.6-2.2)	0.59		
*Adjusted for Age, Charlson comorbidity score, cephalosporins, fluoroquinolones and azithromycin						

The multivariate model that included fluoroquinolones, cephalosporins and azithromycin, in addition to several potentially confounding demographic and clinical variable including age and CCI demonstrated a significant association between fluoroquinolones exposure (OR 2.4, 95% CI 1.4-4.4) and macrolide exposure (4.7, 95% CI 1.1-20.5) with resistance (Tables 3.3). A similar analysis was done for multidrug resistant isolates and is shown in Appendix 5.2.3.

3.3.3 Antimicrobial Resistance and Clinical Outcomes

There were 98 (26.4%) patients who developed the composite outcome, 28 (21.8%) in the no resistance group and 70 (28.8%) in the resistance group (Table 3.4). There were no differences noted between the development of the composite outcome and resistance in both the unadjusted and multivariable adjusted models (Table 3.5)

Table 3.4 Summary of the counts of the composite outcome and secondary outcomes

	No resistance (n = 169)	At least one antimicrobial resistance (n = 341)	Total (n = 510)
Primary outcome			
Composite outcome, n (%) [a]	28 (16.6)	70 (20.5)	98 (19.2)
Secondary outcomes			
Failure to achieve clinical cure, n (%)	6 (3.6)	28 (8.2)	34 (6.7)
30 day relapse, n (%) [b]	16 (10.2)	37 (12.0)	53 (11.4)
90 day relapse, n (%) [c]	9 (6.4)	11 (4.1)	20 (4.9)
CDI-related Death, n (%)	6 (3.7)	5 (1.6)	11 (2.3)

[a] Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI
[b] n after exclusion of patients who failed to achieve clinical cure and death
[c] n after exclusion who failed to achieve clinical cure, death and 30 day relapse

Table 3.5 Summary of unadjusted and adjusted cause specific proportional hazard model				
	Composite outcome*		Other causes of death	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Unadjusted model				
Resistance	1.4 (0.9-2.1)	0.17	2.4 (0.7-9.0)	0.18
Adjusted model**				
Resistance	1.2 (0.8-1.9)	0.42	1.8 (0.5-6.6)	0.36
*Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI **Adjusted for age, ICU stay, toxin test and treatment				

3.3.4 Resistance and Ribotypes

There were a total 498 samples that were ribotyped successfully. The most common ribotype identified was 027 followed by 014-020 (Figure 3.1). Almost all 027 ribotype isolates had antimicrobial resistance detected (n =87, 92.9%) followed by ribotype 106 (n =49, 72.1%) (Figure 3.2). Multidrug resistance was most commonly detected in ribotype 027 (33 isolates, 58.9%). Moxifloxacin, rifampin and clindamycin resistance were most frequently identified.

Ribotype 027 was associated with 21 (31.8%) cases of the composite outcome, followed by ribotypes 014-020 (n=12, 22%) and 106 (n=12, 23.5%) detailed in Table 3.6.

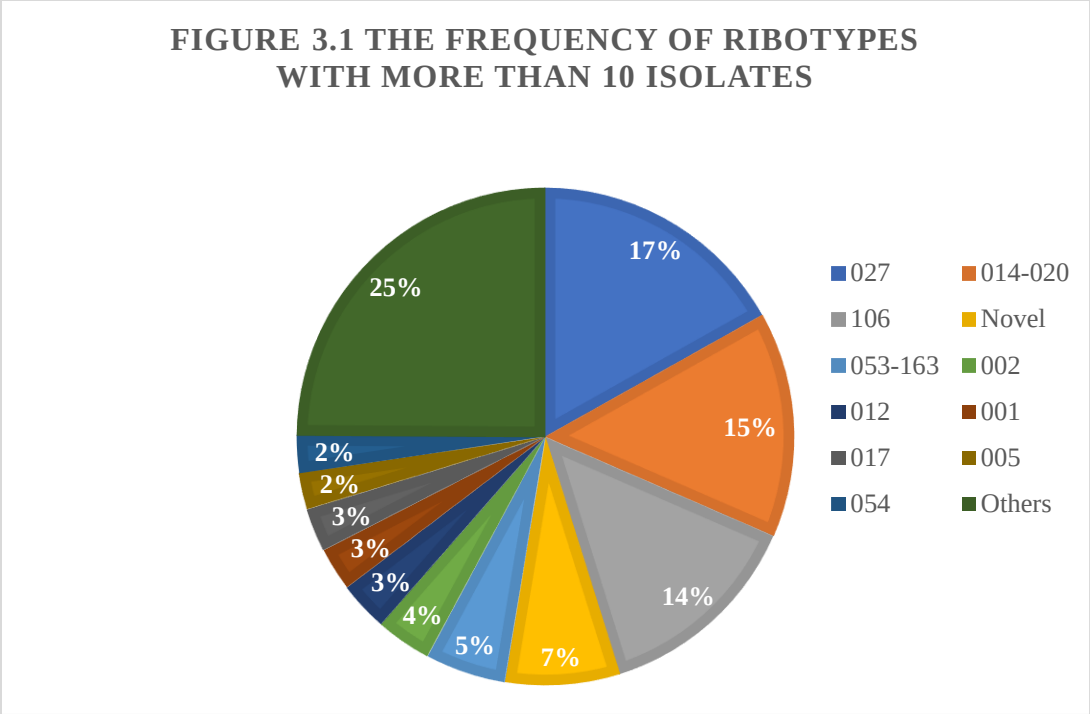

Figure 3.1 The frequency of Ribotypes with more than 10 isolates

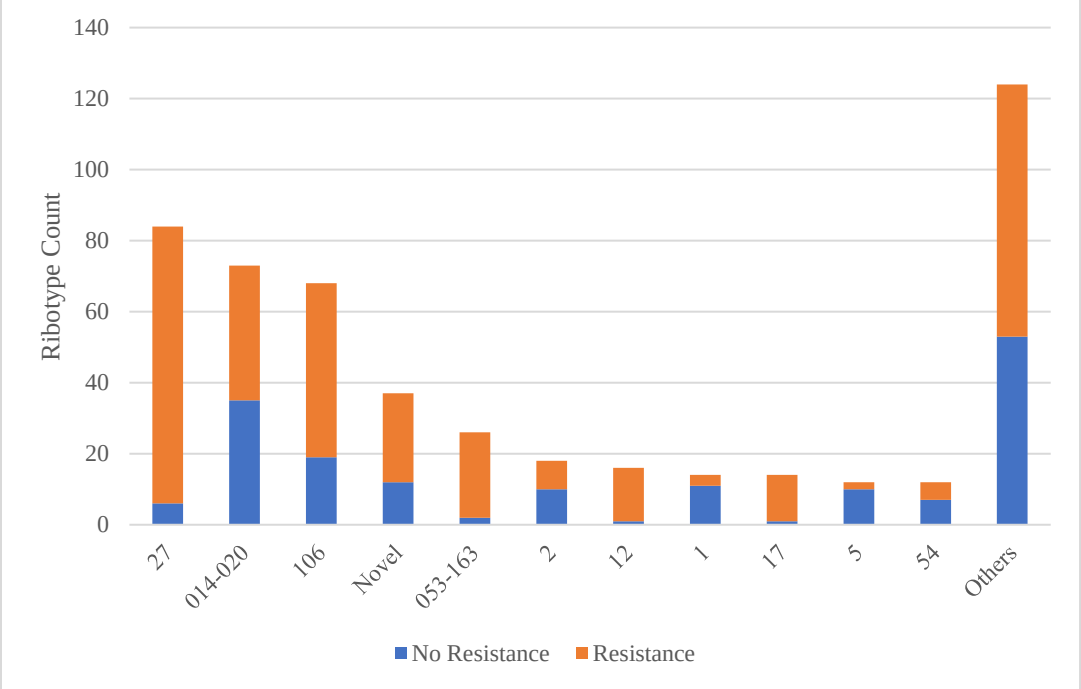

Figure 3.2 The presence of at least one antibiotic resistance by ribotype

Table 3.6 Summary of the counts and percentages of the composite outcome by ribotype			
	Composite outcome (n=98)	No composite outcome (n=273)	Total ribotype
Ribotype 027, n (%)	21 (31.8)	45 (68.2)	66
Ribotype 014-020, n (%)	12 (22.2)	42 (77.8)	54
Ribotype 106, n (%)	12 (23.5)	39 (76.5)	51
Ribotype 053-163, n (%)	10 (52.6)	9 (47.4)	19
Ribotye 2, n (%)	2 (16.7)	10 (83.3)	12
Ribotype 12, n (%)	2 (20.0)	8 (80.0)	10
Ribotype 17, n (%)	3 (37.5)	5 (62.5)	8
Ribotype 5, n (%)	2 (25.0)	6 (75.0)	8
Ribotype 54, n (%)	4 (50.0)	4 (50.0)	8
Other ribotypes, n (%)	18 (21.4)	66 (78.6)	84

3.4 Discussion

The present study results support the notion that prior antimicrobial exposure specifically to fluoroquinolones is implicated in increased resistance. Fluoroquinolone exposure was associated with more than twice the odds of isolating resistant and multidrug resistant *C.difficile* isolates while exposure to azithromycin was also associated with markedly increased odds of resistance.

We did not find a significant difference in the composite outcome based on resistance. This may be due to the composition of our sample. Fluoroquinolone resistance, typically associated with ribotype 027, has been linked to worse clinical outcomes especially in the setting of outbreaks (58, 66). In this study, ribotype 027

composed only 17% of the total isolates and 24% of the resistant ones, which makes our study different from those previously published in the literature on clinical outcomes.

The importance of *C.difficile* resistance testing is not only related to the potential increased risk of infection but is also necessary to monitor the emergence of resistance to CDI directed therapies. There are a limited number of antimicrobials used for the treatment of CDI, which include fidaxomicin, vancomycin and metronidazole. Only 34 (6.7%) isolates were resistant to vancomycin (MIC = 4) and 10 (2%) isolates were resistant to metronidazole (MIC = 4) based on EUCAST. None of the isolates met CLSI resistance breakpoints for vancomycin or metronidazole. There was no resistance to fidaxomicin noted. Our resistance rates were similar to others reported in the literature, a recent meta-analysis published in 2020 found vancomycin be resistant in 3.7% of isolates while metronidazole was resistant in 3.2% (67).

In our study, antimicrobial resistance was predominately detected in ribotypes 027, 106 and 014-020. The composite outcome was mostly seen in ribotypes 027, 014-020 and 106. The association between ribotype and clinical outcomes has been established for a few ribotypes. There is evidence of increased risk of recurrence with ribotype 027 compared to other ribotypes (68, 69). Further, there are fewer data on clinical outcomes following infection with ribotypes 014-020 and 106. Ribotype 106 has been linked to less severe disease than ribotype 027 but potentially increased risk of recurrence; however, the study was limited by small sample size (70). On the other hand, ribotype 014-020 was previously associated with better clinical outcomes compared to ribotype 027 (71, 72).

Strengths of the current study include riboype and resistance data that were collected in an endemic setting with lower rates of ribotype 027. In addition, clinical and demographic information were used to describe selection of *C.difficile* isolates and correlation with clinical outcomes. There are several limitations to this study. First, this was a retrospective, observational, single center study design which creates bias and limits generalizability. Another potential limitation is that we only included one sample per patient and did not include samples from subsequent relapses or episodes.

In conclusion, *C.difficile* antimicrobial resistance is widespread even in endemic settings. The use of fluoroquinolones and macrolides increases the development of resistant and multidrug resistant strains. We did not observe differences in poor outcomes in the presence of resistance. Larger cohorts are needed in similar endemic settings to further explore these findings.

3.5 Student contribution

Majd Alsoubani performed the literature review, statistical analysis and wrote all aspects of the chapter.

Chapter 4: Extended discussion

The risk of developing *C.difficile* infection and recurrence is multifactorial. There are patient risk factors and *C.difficile* strain and virulence factors that make prevention and treatment challenging. In this study, we evaluated several risk factors for CDI recurrence and treatment failure. First, we evaluated the clinical effectiveness of treatment using fidaxomicin versus vancomycin in the immunocompromised population. Second, we evaluated the impact of antecedent antimicrobial exposure on *C.difficile* resistance and the effect of resistance isolation on clinical outcomes.

4.1 The Comparative Effectiveness of Fidaxomicin Compared to Vancomycin in Immunocompromised Patients

In the first part of the study, we evaluated the use of fidaxomicin versus vancomycin in patients with immunocompromising conditions. We chose a composite outcome of failure to achieve clinical cure, relapse at 30 days from treatment completion and CDI-related death. The components of the composite outcome were chosen as representatives of treatment failure. We showed that fidaxomicin use in patients with immunocompromising conditions who are at increased risk of CDI recurrence and complications, was associated with a significant 65% reduction in treatment failure, accounting for the competing risk of death from other causes .This patient population is often unrepresented in clinical trials. The randomized clinical trials for CDI treatment excluded patients with solid or hematologic transplants and were limited by the number of other immunocompromised patients (17, 19).

A previous study in patients with HSCT did not show a significant difference in clinical cure and recurrence between fidaxomicin and vancomycin but the study was

limited by a sample size of less than 100 and a limited number of outcomes (22). On the other hand, a study in cancer patients, demonstrated significantly lower recurrence rates in the fidaxomicin group (21). Thus, the findings of this study add to the body of evidence in this population. Identifying optimal management in patients with increased risk of recurrence, not only decreases the risk of CDI related complications and health care costs, but also improves quality-of-life metrics for patients. An additional advantage to using fidaxomicin is a narrower spectrum of activity compared to vancomycin or metronidazole which helps preserve the intestinal microbiome (73).

Fidaxomicin usage was lower than we expected in this cohort given our institutional recommendations to use fidaxomicin in patients with increased risk of recurrence including those with immunocompromising conditions. We engaged several stakeholders to explore these findings. First, we engaged the antimicrobial stewardship team who updates and disseminates hospital wide recommendations for the treatment of infectious diseases including CDI. The team includes infectious disease specialists and pharmacists. We also engaged members of the gastroenterology and infectious disease teams as key stakeholders who are typically involved in CDI treatment prescription and management. They identified several challenges to prescribing fidaxomicin including cost, insurance coverage and lack of data in patients with immunocompromising conditions. Although, a few cost effectiveness studies showed that fidaxomicin was associated with similar cost to vancomycin when used as first line in the general population, these studies are typically influenced by the rate of recurrence and prevalence of the disease (74, 75).

4.2 Clostridioides difficile resistance

The second part of the study evaluated the patterns of *C.difficile* resistance, ribotypes and clinical outcomes. In our study, two thirds of isolates were resistant to at least one antibiotic. Resistance was predominantly to rifampin, moxifloxacin and clindamycin which was consistent with previous reports (67). Fluoroquinolone was associated with twice the odds of isolating resistance whereas azithromycin exposure was associated with 4 fold increased odds of isolating resistant *C.difficile* isolates.

The emergence of *C.difficile* that are resistant to multiple antimicrobials, like the NAP1/B1/027 strain, leads to challenges in *C.difficile* treatment and prevention. The detection of increased resistance in the hypervirulent strain NAP1/B1/027 may have provided a selective advantage that contributed to the spread of the strain in the 2000s and, increasingly, reports of failed treatment with vancomycin and metronidazole (11, 55, 58). In our cohort, multidrug resistance, defined as resistance to more than two agents from different classes, was uncommon. The incidence of multidrug resistance has ranged from 25-100% in published studies (76). The current finding of relatively low resistance in this sample may be due to the lower prevalence of ribotypes 027 and 078 in this sample which are associated with multidrug resistance (14).

Fidaxomicin is first line for the treatment of CDI followed by vancomycin as second line. Metronidazole is no longer recommended for the treatment of CDI. Fidaxomicin remains highly active against *C.difficile* (67). In our sample, there was no evidence of fidaxomicin resistance. Similar to previously published reports, we identified low rates of vancomycin and metronidazole resistance in our sample (62).

Similar to the first part of the study, a composite outcome was used to denote treatment failure. We showed that there was no association between the presence of resistance and the composite outcome. Fluoroquinolone resistance, which is typically associated with ribotype 027, has been linked to increased risk of recurrence (55, 77). The prevalence of ribotype 027 was low in our sample which may explain the lack of association.

In conclusion, our study showed that the use of fidaxomicin appears to be more effective than vancomycin in treating CDI and preventing poor outcomes. This study contributes to the limited literature of CDI in patients with immunocompromising conditions. Further, upon exploring the relationship between *C.difficile* resistance and clinical outcomes, there were no significant differences.

The evaluation of the effect of *C.difficile* resistance and ribotypes on clinical outcomes especially in populations with immunocompromising conditions can potentially help tailor and individualize CDI directed therapy and non-CDI antibiotics to optimize clinical cure and decrease the risk of recurrence. Future studies should consider including *C.difficile* strain specific factors when evaluating clinical outcomes.

4.3 Student contribution

Majd Alsoubani performed the literature review and wrote all aspects of the chapter.

Chapter 5: Appendix

5.1: Supplemental Materials for Chapter 2

Table 5.1 List of immunosuppressive and immunomodulator agents	
Infliximab	Azathioprine
Tocilizumab	Basliximab
6-mercaptopurine	Cyclophosphamide
Cyclosporine	Imatinib
Adalimumab	Ixekizumab
Vedolizumab	Leflunomide
Nivolumab	Sorafenib
Tofacitinib	

5.1.1 Excluded Patient Data Analysis

Patients who did not receive treatment for CDI were excluded from the main analysis. There was concern of including patients who tested positive for *C.difficile* in the setting of colonization. Patients who did not receive treatment were less likely to have been tested in the inpatient setting (56.3% vs 85.2%, p = 0.008) and to have had a positive toxin assay (29.4% vs 56.8%, p = 0.04) which could point towards less severe disease or colonization. Additional differences included in Appendix Table 5.2.

We excluded patients who received metronidazole alone as treatment for CDI given recent changes in the IDSA guidelines, concerns of decreased efficacy compared to vancomycin and fidaxomicin and use in less critically ill patients. As noted in Appendix Table 5.3, patients who received metronidazole were less likely to be in the ICU (4.8% vs

17.7%, p = 0.04) and less likely to have been diagnosed inpatient (60.5% vs 85.2%, p = <0.001).

Table 5.2 Demographic and clinical characteristics of patients excluded for receiving no CDI directed therapy compared to included patients [a]

	No treatment received (n = 17)	Treatment with vancomycin or fidaxomicin (n = 238)	p-value
Age, Median (Q1,Q3) [b]	64.6 (55.2, 72.6)	63.5 (54.5,71.5)	0.96
Male, n (%)	9 (52.9)	125 (52.5)	0.97
White, n (%)	12 (70.6)	173 (75.2)	0.77
Hispanic, n (%)	0	13 (5.7)	0.61
Type of immunosuppression, n (%)			0.051
SOT	3 (17.6)	76 (31.9)	
BMT	0 (0.0)	24 (10.1)	
Leukemia or Lymphoma	2 (11.8)	48 (20.2)	
Solid Tumor on active chemotherapy	4 (23.5)	44 (18.5)	
Immunomodulation	8 (47.1)	46 (19.3)	
Charlson co-morbidity index, Median (Q1,Q3) [b]	5 (3,9)	5 (3,8)	0.50
Severe CDI, n (%)	3 (23.1)	87 (39.4)	0.24
ICU stay, n (%)	0 (0.0)	40 (17.7)	0.36
WBC, Median (Q1,Q3)[b]	9200 (6500,19600)	7500 (4100,12600)	0.21
Location prior to diagnosis, n (%)			0.51
Hospital acquired	4 (28.6)	101 (43.3)	
Health care associated	6 (42.9)	68 (29.2)	
Community acquired	4 (28.6)	64 (27.5)	
Inpatient testing, n (%)	9 (56.3)	195 (85.2)	0.008
Toxin Test, n (%)	5 (29.4)	130 (56.8)	0.04
Antecedent antibiotic exposure, n (%)	11 (64.7)	172 (75.1)	0.39
Antibiotics during treatment, n (%)	4 (23.5)	123 (51.7)	0.04

a Chi-square test unless otherwise specified
b Mann U Whitney test

Table 5.3 Demographic and clinical characteristics of patients excluded for receiving metronidazole therapy compared to included patients [a]

	Metronidazole (n = 43)	Treatment with vancomycin or fidaxomicin (n = 238)	p-value
Age, Median (Q1,Q3) [b]	59.9 (45.7, 74.3)	63.5 (54.5, 63.5)	0.38
Male, n (%)	20 (46.5)	125 (52.5)	0.51
White, n (%)	28 (65.1)	173 (75.2)	0.19
Hispanic, n (%)	2 (4.7)	13 (5.7)	0.99
Type of immunosuppression, n (%)			0.11
SOT	7 (16.3)	76 (31.9)	
BMT	3 (7.0)	24 (10.1)	
Leukemia or Lymphoma	10 (23.3)	48 (20.2)	
Solid Tumor on active chemotherapy	8 (18.6)	44 (18.5)	
Immunomodulation	15 (34.9)	46 (19.3)	
Charlson co-morbidity index, Median (Q1,Q3) [b]	6 (3,8)	5 (3,8)	0.45
Severe CDI, n (%)	11 (33.3)	87 (39.4)	0.57
ICU stay, n (%)	2 (4.8)	40 (17.7)	0.04
WBC, Median (Q1,Q3) [b]	6300 (3500,11500)	7500 (4100,12600)	0.37
Location prior to diagnosis, n (%)			0.16
Hospital acquired	14 (32.6)	101 (43.3)	
Health care associated	11 (25.6)	68 (29.2)	
Community acquired	18 (41.9)	64 (27.5)	
Inpatient testing, n (%)	26 (60.5)	195 (85.2)	<0.001
Toxin Test, n (%)	22 (51.2)	130 (56.8)	0.51
Antecedent antibiotic exposure, n (%)	22 (55.0)	172 (75.1)	0.01
Antibiotics during treatment, n (%)	18 (42.9)	123 (51.7)	0.32

a Chi-square test unless otherwise specified
b Mann U Whitney test

5.1.2 Loss of Follow up and Missing Data Analysis

The variable with the most missing data (7%) was severity of CDI due to missing WBC results. Patients with loss of follow up were comparable to those without loss of follow up except for age and race. Patients who were lost to follow up were older and more likely to be white. However, there were no differences in markers of severity including, ICU stay, location of testing or toxin positivity.

Table 5.4 The counts and percentages of missing data by variable

Variable	Missing count (n = 238)
Severity of CDI (%)	17 (7.1)
ICU stay (%)	12 (5.0)
Antecedent antibiotics (%)	9 (3.8)
Test type (%)	9 (3.8)
Location of testing (%)	9 (3.8)
Ethnicity (%)	9 (3.8)
Race (%)	8 (3.4)
Location prior to diagnosis (%)	8 (2.8)
Prior CDI diagnosis within 6 months (%)	5 (2.1)
IBD (%)	5 (2.1)
Antibiotics during treatment (%)	2 (0.8)
Gastric Acid (%)	1 (0.4)
IBD: inflammatory bowel disease	

The median censored time for those who were lost to follow up was 20 days (QI 11.5, 34.0). Appendix Table 5.5 displays characteristics of patients by loss of follow up.

Table 5.5 Demographic and clinical characteristics of patients by loss of follow up [a]			
	Loss of follow up (n = 40)	No loss of follow up (n = 198)	p-value
Age, Median (Q1,Q3) [b]	66.9 (57.6,73.7)	62.7 (53.5,71.1)	0.04
Male, n (%)	21 (52.5)	104 (52.5)	0.99
White, n (%)	33 (86.8)	140 (72.9)	0.07
Hispanic, n (%)	0 (0.0)	13 (6.8)	0.13
Type of immunosuppression, n (%)			0.40
SOT	9 (22.5)	67 (33.8)	
BMT	3 (7.5)	21 (10.6)	
Leukemia or Lymphoma	8 (20.0)	40 (20.2)	
Solid Tumor on active chemotherapy	11 (27.5)	33 (16.7)	
Immunomodulation, n	9 (22.5)	37 (18.7)	
CCI (Q1,Q3) [b]	6 (4,9)	5 (3,8)	0.35
Severe CDI, n (%)	12 (34.3)	75 (40.3)	0.50
ICU, n (%)	4 (10.3)	36 (19.3)	0.18
WBC Median (Q1,Q3) [b]	8100.0 (3900,12100)	7400.0 (4150,12825)	0.76
Location prior to diagnosis, n (%)			0.79
Hospital acquired	15 (38.5)	86 (44.3)	
Health care associated	12 (30.8)	56 (28.9)	
Community acquired	12 (30.8)	52 (26.8)	
Inpatient testing, n (%)	31 (79.5)	164 (86.3)	0.28
Toxin Test, n (%)	19 (47.5)	111 (58.7)	0.19
Antecedent antibiotic exposure, n (%)	28 (73.7)	144 (75.4)	0.82
Antibiotics during treatment, n	20 (50.0)	103 (52.0)	0.82
Fidaxomicin, n	5 (12.5)	33 (16.7)	0.64
a Chi-square test unless otherwise specified, b Mann U Whitney test			

5.1.3 Entire Population Analysis of Both Immunocompromised and Immunocompetent Hosts

Table 5.6 Demographic and clinical characteristics of entire patient population by treatment [a]			
	Fidaxomicin (n=87)	Vancomycin (n=550)	p-value
Age, Median (Q1,Q3) [b]	68.2 (68.2, 76.7)	64.9 (53.9, 73.1)	0.07
Male, n (%)	33 (37.9)	282 (51.3)	0.02
White, n (%)	71 (81.1)	407 (73.9)	0.16
Hispanic, n (%)	4 (4.8)	28 (5.1)	0.89
Immunocompromised, n (%)	200 (36.6)	38 (43.7)	0.21
CCI, Median (Q1, Q3) [b]	5.0 (3,7)	5.0 (3,7)	0.06
Dialysis, n (%)	13 (14.9)	45 (8.2)	0.04
CDI in past 6 months, n (%)	7 (8.0)	10 (1.9)	<0.001
Gastric acid suppression, n (%)	56 (64.4)	298 (54.2)	0.07
Location prior to diagnosis, n (%)			
Hospital acquired	50 (57.9)	275 (50.0)	0.28
Health care associated	20 (23.3)	127 (23.1)	
Community acquired	16 (18.7)	148 (26.9)	
Inpatient diagnosis, n (%)	84 (96.4)	476 (86.5)	0.009
Toxin test, n (%)	40 (45.6)	301 (54.8)	0.06
WBC count, Median (Q1,Q3) [b]	9450.0 (6350, 14825)	10900.0 (6800, 17250)	0.31
Severe CDI, n (%)	43 (49.1)	254 (46.2)	0.50
ICU stay, n (%)	22 (25.4)	155 (28.2)	0.40
Antecedent antibiotic use, n (%)	65 (74.3)	411 (74.4)	0.99

Antibiotics during treatment, n (%)	65 (74.3)	411 (74.7)	0.20
Number of antecedent antibiotics, Median (Q1,Q3) [b]	2 (0,3)	1 (1,2)	0.30
Abbreviations: Q1 =interquartile 1, Q3 = interquartile 3, CCI =Charlson comorbidity index, WBC = white blood cell count, ICU = intensive care unit			
[a] Chi-square was used for all testing unless otherwise specified. [b] Mann-U-Whitney			

A total of 844 patient were diagnosed with CDI during the study period. 207 patients were excluded from the analysis of which 159 patients received metronidazole, 2 received fecal microbiota transplant and 46 did not receive any CDI directed therapy, resulting in a total of 637 patients.

Table 5.7 Summary of the counts of the primary and secondary outcomes in patients with and without immunocompromising conditions

	Fidaxomicin (n=87)	Vancomycin (n=550)	Total (n = 637)
Primary outcome			
Composite outcome, n [a] (%)	13 (14.9)	139 (25.3)	152 (23.9)
Secondary outcomes			
Failure to achieve clinical cure n, (%)	10 (11.8)	53 (10.1)	63 (10.4)
30 day relapse, n [b] (%)	2 (2.6)	72 (14.9)	74 (13.2)
90 day relapse, n [c] (%)	4 (5.4)	27 (6.6)	31 (6.4)
Total relapse, n (%)	6 (10.9)	96 (26.9)	102 (24.8)
Death related to CDI, n (%)	1 (1.3)	14 (3.0)	15 (2.8)
[a] Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI [b] n after exclusion of patients who failed to achieve clinical cure and death [c] n after exclusion patients who failed to achieve clinical cure, death and 30 day relapse			

Patients who received fidaxomicin were more likely to be female, have history of prior CDI and on dialysis compared to those who received vancomycin. Appendix Table 5.6 displays patient characteristics by treatment.

The composite outcome occurred in 13 (14.9%) of patients in the fidaxomicin group compared to 139 (25.3%) of patients in the vancomycin group (Appendix Table 5.7).

Table 5.8 Summary of unadjusted and adjusted cause specific proportional hazard model for the entire population

	Composite outcome [a]		Other causes of death	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
All patients				
Unadjusted model				
Fidaxomicin	0.6 (0.3-1.1)	0.07	3.0 (1.2-7.4)	0.02
Adjusted model [b]				
Fidaxomicin	0.5 (0.3-0.9)	0.03	2.6 (1.1-6.5)	0.04
By Immunocompromised Status				
Unadjusted model [c]				
Fidaxomicin-immunocompromised	0.5 (0.2-1.3)	0.18	3.7 (1.2-11.7)	0.03
Fidaxomicin-not immunocompromised	0.7 (0.3-1.4)	0.27	1.9 (0.4-9.1)	0.41
Adjusted model [d]				
Fidaxomicin-immunocompromised	0.4 (0.1-1.1)	0.07	2.9 (0.9-9.6)	0.07
Fidaxomicin-not immunocompromised	0.7 (0.3-1.4)	0.25	1.9 (0.4-8.9)	0.43

[a] Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI
[b] Adjusted for sex, number of antecedent antibiotics, antibiotics during treatment and severity
[c] Interaction p-value for composite in unadjusted model= 0.70, other death = 0.51
[d] Interaction p-value for composite in adjusted model= 0.41, other death = 0.65

In the entire patient cohort including patients with and without immunocompromising conditions, the adjusted hazard ratio was significantly lower in the fidaxomicin group compared to the vancomycin group (HR =0.5, 95% CI 0.3-0.99). The inclusion of immunocompromised status as an interaction term was not significant. In terms of CDI unrelated death, there was a significantly increased risk of death of other causes in the fidaxomicin group compared to vancomycin even after adjustment. However, after evaluating the causes of death (Appendix Table 5.9), death was related to chronic underlying conditions and did not appear to be clinically related to fidaxomicin use.

Table 5.9 Other causes of death by treatment group in both immunocompromised and immunocompetent patients

Causes of death	Fidaxomicin n = 15	Vancomycin n = 7
Comfort measures due to overall poor cancer prognosis	2	5
Comfort measures due to overall poor neurologic prognosis	4	0
Cardiac arrest in the setting of known cardiac and pulmonary conditions	2	0
Comfort measures due to bleeding erosive esophagitis	0	1
Comfort measures due to persistent pneumothorax in the setting of tuberculosis	0	1
Comfort measures due to end stage heart and lung disease	6	0
Complications post op	1	0

There were 37 patients in the vancomycin group who received longer treatment than the fidaxomicin group creating immortal time bias. Those patients were excluded from the analysis in Appendix Table 5.10. In this analysis, fidaxomicin use was

significantly associated with reduced adjusted hazard of the composite outcome by 50% compared to vancomycin group. There was no significant relationship with other cause of death after adjustment. The inclusion of immunocompromised status as an interaction term was not significant.

Table 5.10 Summary of unadjusted and adjusted cause specific proportional hazard model for the entire population excluding patients treated for more than 30 days

	Composite outcome [a]		Other causes of death	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
All patients				
Unadjusted model				
Fidaxomicin	0.6 (0.3-1.01)	0.051	2.8 (1.1-6.8)	0.03
Adjusted model [b]				
Fidaxomicin	0.5 (0.3-0.9)	0.02	2.4 (0.95-6.0)	0.06
By Immunocompromised Status				
Unadjusted model [c]				
Fidaxomicin-immunocompromised	0.5 (0.2-1.3)	0.16	3.5 (1.1-11.0)	0.04
Fidaxomicin-not immunocompromised	0.6 (0.3-1.3)	0.22	1.7 (0.4-8.2)	0.47
Adjusted model [d]				
Fidaxomicin-immunocompromised	0.4 (0.1-1.03)	0.06	2.8 (.9-9.1)	0.09
Fidaxomicin-not immunocompromised	0.6 (0.3-1.3)	0.22	1.7 (0.4-7.9)	0.52

[a] Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI

[b] Adjusted for sex, number of antecedent antibiotics, antibiotics during treatment and severity

[c] Interaction p-value for composite in unadjusted model= 0.72, other death = 0.54

[d] Interaction p-value for composite in adjusted model= 0.41, other death = 0.60

5.1.4: Other Cause of Death in the Immunocompromised Population

Table 5.11 Cause of death by treatment group in the immunocompromised population		
Causes of death	Fidaxomicin (6)	Vancomycin (11)
CDI unrelated	5	7
CDI related	1	4

Table 5.12 CDI unrelated causes of death by treatment group in the immunocompromised population		
Cause of death	Fidaxomicin n = 5	Vancomycin n = 7
Comfort measures due to overall poor cancer prognosis	2	5
Comfort measures due to overall poor neurologic prognosis	2	0
Cardiac arrest	1	0
Comfort measures due to bleeding erosive esophagitis	0	1
Comfort measures due to persistent pneumothorax in the setting of tuberculosis	0	1

5.1.5: Intention to Treat Sensitivity Analysis

In the primary analysis, treatment was based on CDI therapy administered for at least 72 hours. In an attempt to emulate the target trial (78), the intention to treat effect was estimated by assessing the relationship between outcome and the first dose of CDI therapy received. There were 39 patients in the fidaxomicin group and 182 patients in the vancomycin group. 7 patients received vancomycin as the first dose then switched to fidaxomicin, while, 9 patients received fidaxomicin as the first dose then switched to vancomycin. 17 patients received metronidazole as first dose and were not included in the sensitivity analysis.

Table 5.13 Summary of unadjusted and adjusted cause specific proportional hazard model intention to treat sensitivity analysis				
	Composite outcome*		Other causes of death	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Unadjusted model				
Fidaxomicin	0.42 (0.15-1.2)	0.09	3.7 (1.1-12.3)	0.03
Adjusted model**				
Fidaxomicin	0.33 (0.11-0.96)	0.04	3.6 (0.80-15.1)	0.90
*Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI **Adjusted for sex, number of antecedent antibiotics, antibiotics during treatment and type of immunosuppression				

5.1.6: Analysis to Assess for Immortal Time Bias

There were 10 patients in the vancomycin group who treated for a longer duration than the fidaxomicin group potentially creating immortal time bias. A sensitivity analysis was conducted after excluding those patients displayed in Appendix Table 5.14. The results of the analysis were similar to those of the main analysis.

Table 5.14 Summary of unadjusted and adjusted cause specific proportional hazard model sensitivity analysis to assess immortal time bias				
	Composite outcome*		Other causes of death	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Unadjusted model				
Fidaxomicin	0.51 (0.2-1.3)	0.20	3.5 (1.1-11.0)	0.04
Adjusted model**				
Fidaxomicin	0.32 (0.1-0.9)	0.04	3.1 (0.8-11.4)	0.09
*Composite outcome: failure to achieve clinical cure, relapse at 30 days and death related to CDI **Adjusted for sex, number of antecedent antibiotics, antibiotics during treatment, severity and type of immunosuppression				

5.2: Supplemental Materials for Chapter 3

Table 5.15 Antimicrobial agents tested and MIC breakpoints		
Antimicrobial	MIC Resistance Breakpoints (µg/ml)	
	CLSI	EUCAST
Fidaxomicin	NA	NA
Rifaximin	NA	NA
Rifampin	NA	>0.004
Vancomycin	NA	>2
Metronidazole	≥32	>2
Moxifloxacin	≥8	>4
Clindamycin	≥8	NA
Imipenem	≥16	NA
Tigecycline	≥16	>0.25
Chloramphenicol	≥32	NA
NA not applicable		

Table 5.16 list of antimicrobial classes	
Aminopenicillins	Amoxicillin, amoxicillin-clavulanate, ampicillin, ampicillin-sulbactam, penicillin, dicloxacillin
Cephalosporins	cefazolin, cefadroxil, cephalexin, cefaclor, cefotetan, cefoxitin, cefidinir, cefpodoxime, ceftazidime, ceftriaxone, cefepime
Fluoroquinolones	ciprofloxacin, levofloxacin, moxifloxacin
Macrolides	erythromycin, azithromycin
Aminoglycosides	amikacin, gentamicin, tobramycin
Tetracyclines	minocycline, doxycycline, tetracycline

5.2.1: Definitions

Index CDI was defined as the first episode of CDI with a documented test in the medical record after the diagnosis of the immunocompromising condition. Patients with previous episodes of CDI as reported in the clinical notes were defined as having history of CDI.

Failure to achieve clinical cure was defined as the presence of persistent diarrhea despite medical treatment as determined by the treating physician within 72 hours of treatment initiation.

Relapse at 30 days was defined as recurrence of CDI or the need to restart CDI treatment within 30 days (+7 days) of stopping therapy for the index CDI case, as determined by the treating physician.

Relapse at 90 days was defined as recurrence of CDI or the need to restart CDI treatment between 30 and 90 days (+7 days) of stopping therapy for the index CDI case, as determined by the treating physician, excluding relapses that occurred before 30 days.

Death related to CDI was defined as any death that was attributed to CDI within 30 days of initial diagnosis. This included death from fulminant colitis or septic shock.

Death from other cause, a competing risk to the primary outcome, was defined as any death that was not associated with CDI within 30 days of CDI diagnosis. This included cardiac, pulmonary or cancer related deaths.

Severe CDI was defined based on IDSA criteria; leukocytosis with white cell count (WBC) of $\geq$ 15,000 cells/ml or serum creatinine (SCr) >1.5 mg/dl (3).

5.2.2: Loss of Follow up and Missing Data Analysis

The variable with the most missing data (10.6%) was severity of CDI due to missing WBC results. Patients with loss of follow up were comparable to those without except for immunocompromised status. Patients who were immunocompromised were less likely to have been lost to follow up.

Table 5.17 The counts and percentages of missing data by variable

Variable	Missingness, n (%) (n=510)
Severity of CDI	54 (10.6)
ICU stay	38 (7.5)
Health care associated infection	21 (4.1)
Antecedent antibiotics	15 (2.9)
Location of testing	14 (2.7)
Prior CDI diagnosis within 6 months	10 (2.0)
Test type	8 (1.6)
CCI	7 (1.4)
Antibiotics during treatment	6 (1.2)
Dialysis	5 (1.0)
Immunocompromised	4 (0.8)
Gastric Acid suppression	2 (0.4)
Ethnicity	2 (0.4)
CCI: Charlson Co-morbidity score, ICU: Intensive care unit	

Table 5.18 Demographic and clinical characteristics of patients by loss of follow up [a]			
	Loss of follow up (n = 139)	No loss of follow up (n = 371)	p-value
Age, Median (Q1,Q3)[b]	66.6 (55.1, 75.2)	63.6 (53.3, 63.6)	0.07
Male, n (%)	72 (51.8)	172 (46.4)	0.27
White, n (%)	109 (78.4)	273 (73.6)	0.30
Hispanic, n (%)	8 (5.8)	15 (4.1)	0.47
Immunocompromised, n (%)	30 (22.1)	128 (34.6)	0.009
CCI, Median (Q1,Q3) [b]	4 (2, 6)	4 (2,6)	0.75
Severe CDI, n (%)	47 (36.7)	147 (44.8)	0.14
ICU stay, n (%)	23 (18.9)	79 (22.6)	0.44
WBC, Median (Q1,Q3) [b]	11100 (6800, 15575)	10400 (6500, 16700)	0.87
Health care associated, n	95 (73.1)	253 (70.5)	
Inpatient testing, n (%)	112 (85.5)	293 (80.3)	0.19
Toxin Test, n (%)	77 (55.4)	204 (56.2)	0.92
Antecedent antibiotic exposure, n (%)	95 (71.4)	271 (74.9)	0.49
Antibiotics during treatment, n (%)	69 (50.0)	205 (56.0)	0.23
The presence of at least one antimicrobial resistance, n (%)	98 (70.5)	243 (65.5)	0.65
a Chi-square test unless otherwise specified b Mann U Whitney test			

5.2.3: Multidrug Resistant Isolates

Patients who had isolates exhibiting multidrug resistance were more likely to be older, had higher CCI median and were more likely to have a positive toxin test.

Table 5.19 Patient demographic and clinical characteristics of *C.difficile* isolates exhibiting multidrug antimicrobial resistance pattern [a,b]			
	No multidrug resistance (n = 452)	Multidrug resistance pattern (n=58)	p-value
Age (yr), Median (Q1,Q3)[c]	63.3 (51.7, 73.0)	66.1 (58.2, 78.2)	0.049
Male, n (%)	234 (51.8)	32 (55.2)	0.68
White, n (%)	338 (74.8)	44 (75.9)	0.86
Hispanic, n (%)	21 (4.6)	2 (3.6)	0.72
Immunocompromised, n (%)	136 (30.1)	22 (37.9)	0.23
CCI, Median (Q1,Q3)[c]	4 (2, 6)	5 (3, 7.5)	0.001
Dialysis, n (%)	27 (6.0)	4 (6.9)	0.80
History of CDI, n (%)	14 (3.0)	2 (3.6)	0.80
Gastric Acid Suppression, n (%)	255 (56.4)	39 (67.2)	0.12
Health care associated, n (%)	313 (69.1)	48 (82.7)	0.04
Inpatient testing, n (%)	367 (81.2)	49 (84.0)	0.61
Toxin, n (%)	244 (54.1)	42 (72.4)	0.009
WBC, Median (Q1,Q3)[c]	10450.0 (6625.0, 16100.0)	11400.0 (5700.0, 18900.0)	0.42
Severe CDI, n (%)	178 (39.3)	29 (49.7)	0.14
ICU, n (%)	102 (22.5)	14 (23.6)	0.86
Antecedent antimicrobial exposure, n (%)	321 (70.9)	55 (94.5)	0.001
Antimicrobials during treatment, n (%)	242 (53.6)	33 (56.9)	0.63
Number of antecedent antimicrobials, Median (Q1,Q3)[c]	1.1 (1.2)	1.5 (1.2)	<0.001
a Multidrug resistance: resistance to >2 antibiotics from different classes, b Chi-square test unless otherwise specified, c Mann U Whitney test			

Table 5.20 Univariate and multivariate analysis of antecedent antibiotic class and the presence of multidrug resistance						
	Multidrug resistance (n = 58)	No multidrug resistance (n=452)	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Aminopenicillins	7 (12.3)	34 (7.8)	1.7 (0.7-3.9)	0.25		
Cephalosporins	27 (47.4)	169 (38.6)	1.4 (0.8-2.5)	0.20		
Carbapenems	13 (22.8)	59 (13.5)	1.9 (0.97-3.7)	0.06	1.7 (0.8-3.4)	0.16
Aminoglycosides	0 (0.0)	4 (0.9)	-	-		
Fluoroquinolones	17 (29.8)	68 (15.5)	2.3 (1.2-4.3)	0.008	2.3 (1.2-4.3)	0.01
Piperacillin-tazobactam	8 (14.0)	39 (8.9)	1.7 (0.7-3.8)	0.22		
Vancomycin	25 (43.9)	131 (29.9)	1.8 (1.0-3.2)	0.04	1.5 (0.8-2.7)	0.20
Metronidazole	3 (5.3)	39 (8.9)	0.6 (0.2-1.9)	0.36		
Azithromycin	8 (14.0)	14 (3.2)	4.9 (2.0-12.4)	<0.001	4.1 (1.6-10.8)	0.004
Clindamycin	0 (0.0)	11 (2.5)	-	-		
Other antibiotics	10 (17.5)	44 (10.0)	1.9 (0.9-4.0)	0.09		
Adjusted for age, Charlson Comobidity score, azithromycin, fluoroquinolones, vancomycin and carbapenems						

5.3: Supplemental Material for the Thesis

5.3.1 Model Building for Chapter 2

5.3.1.1 Collapsibility Approach and Clinical Knowledge

The collapsibility approach was used to determine candidate variables to be included in the cause specific Cox proportional hazard model to adjust for confounders.

The variables were determined *a priori* and collected from the medical charts based on previously published literature and clinical knowledge. Variables were considered in the model if there was a change in beta coefficient of more than 20%. Type of immunosuppression and antibiotics during treatment were added to the model due to clinical significance (79).

5.3.1.2 Proportional Hazard Assumptions

The proportional hazard assumptions were tested using the log(-log) plot versus time for every categorical variable included in the model as shown in Appendix Figure 5.1. Parallel lines indicate proportionality since proportional hazard functions imply that the log differs at a constant rate for the different groups. Two variables showed overlap including type of immunosuppression and severity of CDI. These variables were further explored by evaluating the graphical representation of Schoenfeld residual plots (39).

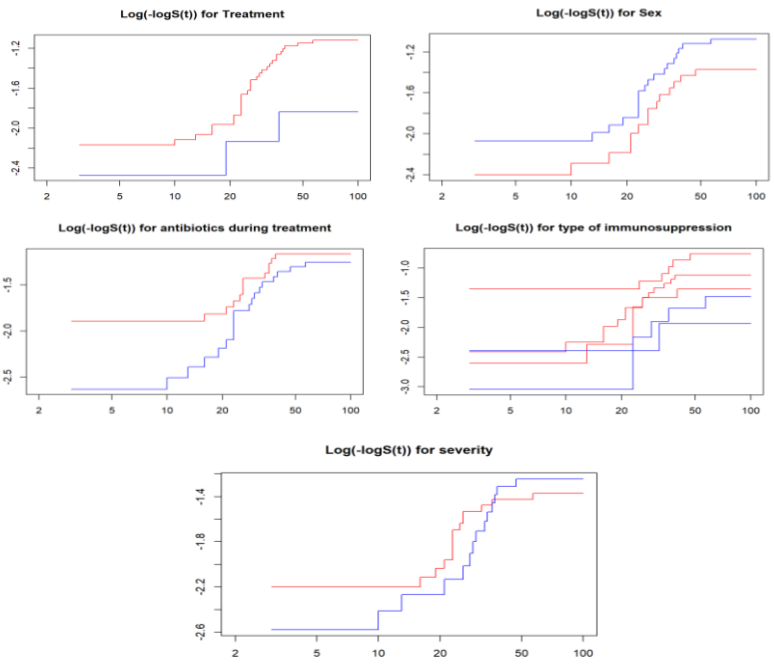

Figure 5.1 Log(-log) plots for categorical variables to assess proportional hazard assumptions

The number of antibiotics prior to treatment was also evaluated. All plots showed a horizontal line fitting between the CI of the plot which indicates proportionality as seen in Appendix Figure 5.2.

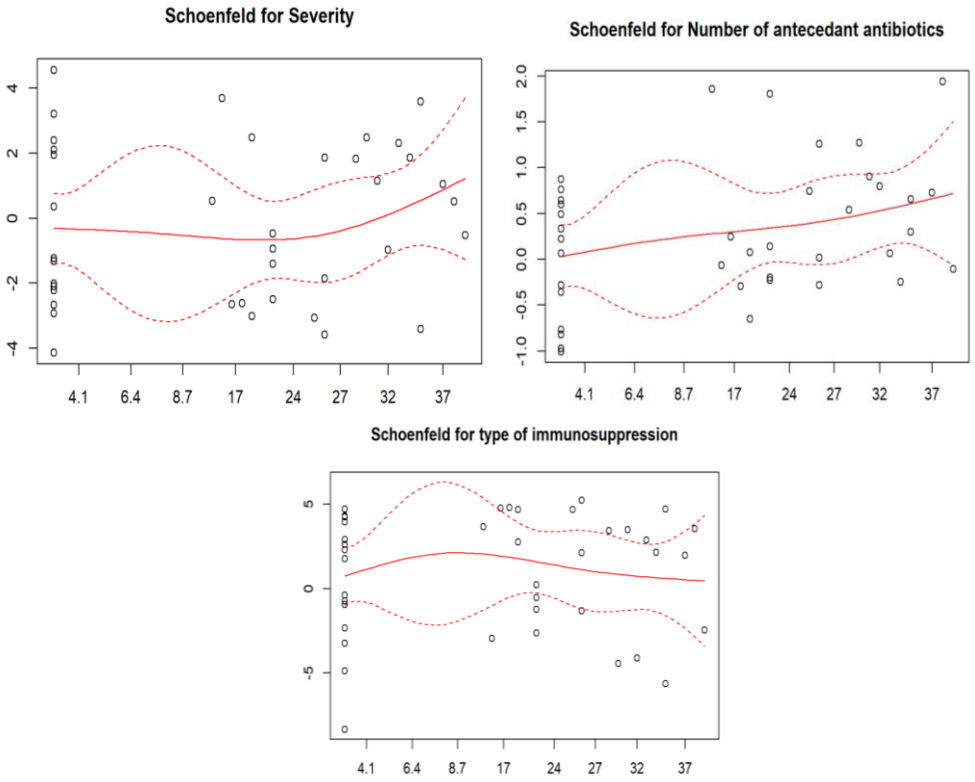

Figure 5.2 Schoenfeld residual plots to assess proportional hazard assumptions

5.3.2 Model Building for Chapter 3

5.3.2.1 Assessment of Collinearity

The variables included in the logistic regression model were checked for collinearity using the correlation matrixes and variance inflation factors (VIF). The correlation matrix including the variables in the model were below 0.8. VIF for each of the variables was below 2 for all variables (80).

5.3.2.2 Univariate Screen and Clinical Knowledge

The univariate screen approach was used to determine candidate variables to be included in the cause specific proportional hazard model (79). The variables were considered in the model if p-value <0.1 in the univariate screen (Appendix Table 5.21).

Table 5.21 Univariate screen of candidate variables based on at least one antimicrobial resistance

	Hazard ratio	95% CI	p-value
Age	1.0	1.00-1.03	0.002
Male	1.2	0.8-1.8	0.31
White	0.6	0.4-1.0	0.07
Hispanic	0.4	0.1-1.7	0.22
Immunocompromised	0.8	0.5-1.2	0.28
Dialysis	0.7	0.3-1.8	0.50
Severity	1.2	0.8-1.9	0.33
Prior CDI in the past 6 months	1.9	0.7-5.0	0.21
Gastric acid suppression	1.0	0.7-1.5	0.92
Health care associated	0.8	0.5-1.2	0.29
Inpatient	0.7	0.4-1.1	0.11
Test type	0.6	0.4-1.0	0.04
CCI	1.1	1.0-1.1	0.04
ICU	1.5	1.0-2.5	0.06
Antecedent antibiotics	0.9	0.6-1.5	0.79
Antibiotics during	0.9	0.6-1.3	0.51
Number of antecedent antibiotics	1.0	0.9-1.2	0.99

5.3.2.3 Assessment of Proportional Hazard Assumptions

The proportional hazard assumptions were tested using the log(-log) plot versus time for every categorical variable included in the model as shown in Appendix Figure 5.3. Parallel lines indicate proportionality since proportional hazard functions imply that the log differs at a constant rate for the different groups (39). Toxin assay and treatment

showed overlap. Further age is a continuous variable, so a log(-log) plot versus time was not created. These variables were further explored by evaluating the graphical representation of Schoenfeld residual plots. All plots showed a horizontal line fitting between the CI of the plot which indicates the model meets the proportionality assumption as seen in Appendix Figure 5.4.

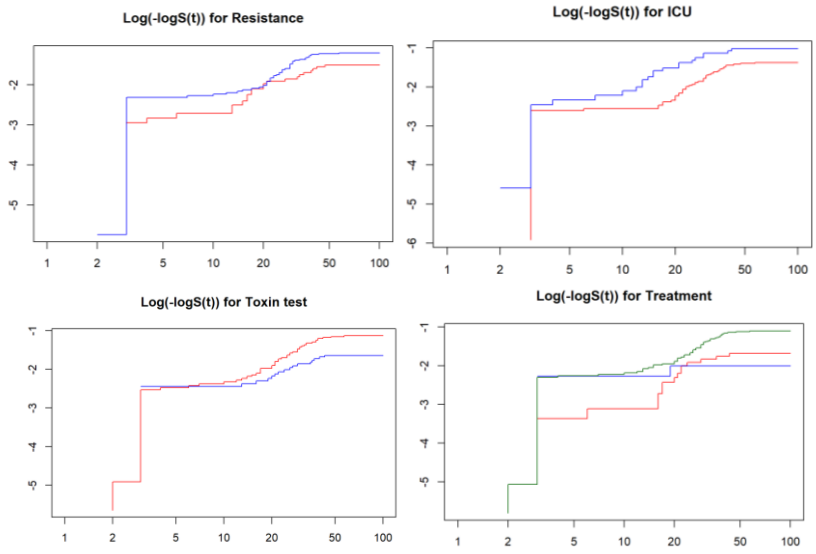

Figure 5.3 Log(-log) plots for categorical variables to assess proportional hazard assumptions

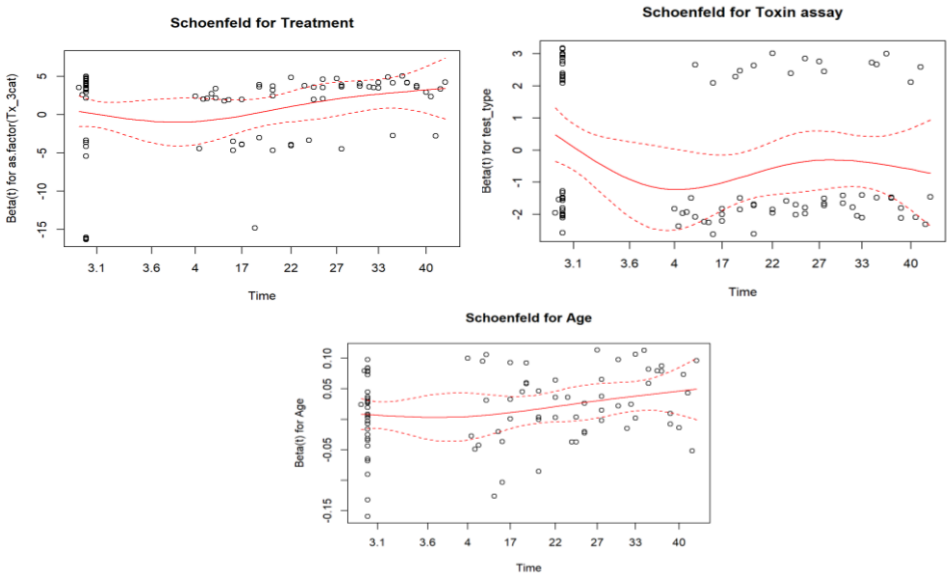

Figure 5.4 Schoenfeld residual plots to assess proportional hazard assumptions

5.3.3 Stakeholder Engagement

The primary stakeholders engaged in this project are providers. Clinicians from different specialties and at different levels of training are responsible for managing and treating patients with CDI. However, gastroenterologists and infectious disease physicians are typically the primary providers. We wanted to understand the perceived barriers to prescribing CDI directed therapy. The initial stakeholders involved were the antimicrobial stewardship committee composed of infectious disease physicians (n = 3, pharmacists (n =2) and infectious disease trainees (n = 3). The role of the committee is to standardize guidelines and protocols for the treatment of common infectious diseases, like CDI. I engaged the stakeholders through discussion about the current institutional CDI treatment guidelines and the perceived barriers to prescribing different agents. The price of fidaxomicin and concern of insurance approval were the primary concerns. A second meeting was held with the gastroenterology team to discuss the optimal treatment for CDI especially for patients with immunocompromising conditions and patients with inflammatory bowel disease. The lack of data on fidaxomicin treatment outcomes in this patient population was noted in the discussions as a barrier to prescribing especially given the high cost of the medication. The pharmaceutical company, Merck, who is the manufacturer of fidaxomicin was involved in the study proposal process. The study findings will be presented to the antimicrobial stewardship committee to determine further changes to the current institutional CDI treatment guidelines.

5.4 Student contribution

Majd Alsoubani performed all statistical analysis and wrote all aspects of the chapter.

Chapter 6: Bibliography

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