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APPROVAL SHEET

Risk Factors for Recurrent *Clostridium difficile* Infection

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ABSTRACT COVER PAGE

Risk Factors for Recurrent *Clostridium difficile* Infection

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M.D., Case Western Reserve University, 2008

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An abstract of

A thesis submitted to the Faculty of the

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ABSTRACT

Risk Factors for Recurrent *Clostridium difficile* Infection

By Sujan C. Reddy

Introduction:
Identifying patients at high risk for recurrent *Clostridium difficile* infection (CDI) is important. Exposures to healthcare facilities and CDI treatment are of particular interest as these factors are readily identifiable and potentially modifiable by clinicians.

Methods:
Population based surveillance for CDI was performed in ten states. Initial CDI cases were defined as an initial positive *C. difficile* test identified between January and December 2013 in a patient with diarrhea or CDI treatment who did not have any previous documented CDI in surveillance. A recurrent case was defined as a positive test over 14 days from the previous positive test in a patient who had diarrhea or CDI treatment for that recurrent positive test. Patients over the age of 17 were included. Patients who did not have documentation of symptoms or treatment were excluded as were patients who died after initial CDI.

Results:
A total of 4,790 adults with initial CDI were included in the analysis. Recurrent CDI was identified in 843 patients (17%). Hospital-onset (HO) CDI was not associated with increased risk of recurrence compared to community associated (CA) CDI in bivariate nor in multivariate analysis ($p>0.05$). In multivariate analysis, factors associated with increased risk of recurrence included: treatment with combination vancomycin and metronidazole, age over 65 years, female sex, white race, hemodialysis use, diabetes, and prior antibiotic use ($p<0.05$). Long term care facility onset (LTCFO) cases had a non-significant increased risk of recurrence compared to community associated disease ($p=0.18$). There was no difference in recurrence risk noted in patients treated with vancomycin compared to those treated with metronidazole ($p=0.23$).

Conclusion:
HO and CA CDI have similar and relatively high risk of recurrence. Recurrence risk among LTCFO cases warrants further study. Treatment with either vancomycin or metronidazole did not affect recurrence risk.

COVER PAGE

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INTRODUCTION

Clostridium difficile is a leading cause of healthcare associated infections and is a significant cause of morbidity and mortality in the United States (1, 2). Clinical disease associated with *C. difficile* infection (CDI) can range from mild diarrhea to severe diarrhea leading to complications such as toxic megacolon and death. Recurrent CDI is common with an estimated 15-30% of all initial CDI cases leading to an additional episode of CDI (3). Furthermore, once a patient experiences a recurrence, they are at higher risk for subsequent recurrences (3).

Exposure to *C. difficile* alone is often not sufficient to cause an infection. The pathophysiology of CDI relies on two predominate factors: exposure to *C. difficile* and disruption of the microbial homeostasis that is normally present in the large intestine, known as the intestinal microbiome. The disruption of the healthy microbiome is called dysbiosis. A number of factors have been associated with intestinal dysbiosis; however antibiotics are thought to be the most common factor in causing dysbiosis and increasing the risk for developing CDI (4). Similarly, recurrent CDI relies on continued dysbiosis and the presence of the pathogen, either the same strain (causing relapse) or from a different strain of *C. difficile* (causing reinfection). Clinically, relapse and reinfections are indistinguishable and are treated in the same manner.

Much attention has focused on where patients may have been exposed to the *C. difficile* bacteria leading to infection (5). Historically, healthcare facility exposures have been considered a major factor in exposing patients to *C. difficile.* However, a significant portion of patients with CDI have no history of overnight stays in healthcare facilities in the preceding 3 months (2). Understanding the routes of exposures is important in reducing transmission events. Furthermore, patients who are in healthcare settings are commonly exposed to agents associated with dysbiosis. Given that exposure to healthcare facilities increases the opportunity for *C. difficile* colonization as well as exposure to agents such as antibiotics that are associated with dysbiosis, we hypothesize that patients with CDI occurring within the hospital (hospital-onset CDI) have higher risk of recurrence than patients with no healthcare exposure (community-associated CDI).

Dysbiosis after initial CDI episodes can be prolonged due to slow recovery of the usual microbiome diversity, the need for ongoing antibiotics for treatment of other infections, host factors that may limit clearance of *C. difficile* from the colon and CDI may directly contribute to continued dysbiosis. Furthermore, the mainstays for treatment of CDI, metronidazole and vancomycin, are both known to cause dysbiosis and are both associated with risk of initial CDI after their use. However, vancomycin is considered to produce less dysbiosis than metronidazole due to its spectrum of microbial activity. Vancomycin has been shown to be superior to metronidazole for treatment of recurrent CDI disease and in those with severe disease (4, 6). A large randomized clinical trial recently showed that vancomycin had better clinical cure rates than metronidazole. However this study did not show a significant difference in risk of recurrence between the two treatments (7). In order to further evaluate this, we assessed the role of treatment on risk of recurrence. Our hypothesis was that initial CDI cases treated with vancomycin would have a lower risk of recurrence than initial CDI cases treated with metronidazole.

BACKGROUND

Significance and Pathophysiology *Clostridium difficile* Infection

Clostridium difficile infection (CDI) is a leading cause of healthcare associated infections (1). In 2011, there was an estimated 453,000 incident cases of CDI with nearly 83,000 recurrences and 29,300 deaths (2). Recurrent disease occurs in 15-30% of all CDI and multiple recurrences are common and can cause significant burden to patients as the cycle of recurrent disease can be difficult to break.

Clostridium difficile is an organism that is commonly found in human intestines. In the 1970s and 1980s, *C. difficile* was identified as a causative agent of antibiotic-associated diarrhea (4, 8). In particular, toxin-producing *C. difficile* was found to cause diarrhea with the potential for complications including toxic megacolon and death. The pathophysiology of CDI relies predominately on two factors: alterations of bacterial homeostasis in the intestines and exposure to the bacterium *C. difficile*. The intestinal microbiome refers to the intricate bacterial homeostasis that develops moments after birth and evolves over time. The intestinal microbiome plays a significant role in nutrition, metabolism and inflammatory states as these organisms are involved in complex biochemical pathways and interact directly with human intestinal cells. Dysbiosis refers to the alteration of this intestinal microbiome. Antibiotic exposure is a significant cause of dysbiosis, which is associated with CDI (4).

Role of Exposures to Healthcare Facilities

Equally important is the exposure to the pathogen. Although dysbiosis can cause self-limiting diarrhea, dysbiosis in the setting of exposure to *C. difficile* can lead to CDI. CDI has been commonly linked to healthcare facilities as patients are at risk of becoming colonized with *C. difficile* during hospitalization (4, 9). *C. difficile* is particularly challenging to eradicate from healthcare environments as the bacterium produces spores that may survive on surfaces for weeks and are less effectively eradicated by certain cleaning methods, particularly alcohol-based hand

sanitizers (10, 11). Although healthcare facilities have been clearly linked to acquisition of *C. difficile*, over the last 10-15 years, a considerable number of CDI cases have occurred in patients with no history of exposure to healthcare facilities (community-associated CDI). In 2011, the incidence of community-associated CDI was 48.2 cases per 100,000 persons compared to 92.8 healthcare facility associated cases per 100,000 persons (2). For epidemiologic purposes, Community-associated (CA) CDI is defined as patients who have not had an overnight stay in a healthcare facility in the last 3 months, whereas healthcare facility-associated (HCFA) disease is defined as patients who have had an overnight stay in a healthcare facility in the last 3 months. HCFA cases can be further divided into those that occur as an outpatient or within 3 days of hospitalization, which are classified as community-onset, healthcare facility associated disease (CO-HCFA). Patients who have been hospitalized for 3 or more days at the time of CDI onset are classified as hospital-onset (HO) or long term care facility onset (LTCFO) depending on whether they were in an acute care (HO) or long term care facility (LTCFO) setting at the time of disease onset.

Recurrent *Clostridium difficile* Infection: Relapse vs. Reinfection

The pathophysiology of recurrent disease is very similar to initial CDI: recurrent disease requires dysbiosis and exposure to *C. difficile*. Often, the exposure to *C. difficile* is a continued exposure to the strain responsible for the initial case; this is called a relapse. Reinfection represents recurrent disease due to a different strain of *C. difficile*. Clinically, relapse CDI and reinfection CDI are indistinguishable by usual clinical microbiology laboratory methods and are treated in a similar fashion. However, the difference between relapse and reinfection may explain some of the variable results from previous studies on recurrent CDI.

As previously stated, reported rates of CDI recurrence vary from 15-30% (3). This variation is likely due to differences in follow-up duration, prevalence of more virulent strains (such as NAP1) and initial treatment regimens. Studies evaluating recurrence utilize follow-up

periods ranging from 30 days to 3 months. The commonly used epidemiologic definition of recurrence is a positive stool assay greater than or equal to two weeks and less than eight weeks after an initial positive result (4). Although most studies have shown that the majority of CDI recurrence occurs within the first eight weeks, a few studies have recorded long-term risk for recurrence (12, 13). Interestingly, one recent study showed that up to 50% of all recurrences occurred after one month (12). Recent strain-typing studies estimate that 65-88% of recurrent CDI is attributable to relapse with the original infecting strain (13). Relapse is particularly more common if the recurrent episode occurs soon after the initial infection. Later episodes of recurrence are equally split between reinfection and relapse (13). Relapse and reinfection differ in the timing of presentation, as one study found that the average number of days to relapse was 40 days and the average number of days to reinfection was 90 days (12). Few previous studies have evaluated longer follow-up time intervals to determine whether any characteristics of the initial CDI episode contributed to the risk of recurrence.

In this setting, we are interested in understanding the role of healthcare exposure on recurrent disease. Readmissions to hospitals are common. As mentioned, hospitalization is a risk factor for *C. difficile* acquisition; therefore, additional healthcare exposure may be a marker for patients at risk for being re-exposed to *C. difficile*. Furthermore, patients who are hospitalized are often exposed to dysbiosis-producing agents such as antibiotics, proton pump inhibitors and immunosuppressive agents. Thus with continued dysbiosis this population is at risk for both relapse and reinfection CDI. Therefore, we aim to assess the role of healthcare facility exposure in identifying patients who are at risk for recurrent disease. Healthcare facility exposure is commonly delineated by epidemiologic classification: CA, CO-HCFA, HO and LTCFO as described above. Patients with community-associated CDI have different clinical characteristics than healthcare-associated CDI, but the risk factors for recurrence in community-associated CDI have not been well elucidated (14, 15). A recent study in one healthcare system found that patients with community-onset, healthcare-associated (CO-HCFA) CDI had higher risk of

recurrence than healthcare-onset cases (16). However the study had few recurrent episodes due to limited outpatient follow-up, which may have biased the result. Our hypothesis is that hospital-onset (HO) cases have a greater risk of recurrence than community-associated (CA) cases.

Risk Factors for Recurrent CDI

A few recent studies have attempted to identify risk factors for recurrent CDI. A meta-analysis of these studies showed that risk factors for recurrence included older age (>65 years old), concomitant administration of non-*C. difficile* antibiotics and use of gastric acid suppressing medications, especially proton-pump inhibitors (17). Other studies have identified additional potential risk factors including underlying disease severity, impaired anti-toxin immune response, prolonged hospital stay, female gender and lymphopenia upon admission (12, 18, 19). Particular co-morbidities have also been associated with increased risk of recurrence including chronic renal insufficiency, immunocompromised states, and inflammatory bowel disease (18). Of note, many of these studies were limited by small sample sizes and insufficient clinical information. Thus attempts to develop clinical prediction tools have been suboptimal and no such tools have been validated (18, 20, 21). Further, few studies were able to evaluate CDI treatment as a potential influence on these risk factors of recurrence.

Role of CDI Treatment on Risk of Recurrence

The effect of CDI treatment on risk of recurrence has been documented in a few randomized, controlled trials, however most have follow-up periods of 30 days or less. Current guidelines recommend treatment of the first episode of CDI with metronidazole for mild to moderate disease, or oral vancomycin for severe disease. In patients with severe complicated disease, in whom delivery of oral vancomycin to the colon is unreliable (such as ileus or toxic megacolon), guidelines recommend treatment with combination intravenous metronidazole and vancomycin (either oral or per rectum) (4). In the available randomized controlled trials, patients

in the metronidazole treatment arms had recurrence rates between 4 and 26% (6, 22-24). Patients in vancomycin treatment arms had recurrence rates between 7 and 28% (24). As mentioned previously, there was significant heterogeneity among the studies with regards to the definition of recurrence (symptom-based definition versus diagnostic test-based) and especially the duration of follow-up (21 days to 6 weeks). In three head-to-head randomized controlled trials comparing vancomycin and metronidazole, there was no significant difference in recurrence rates between the two groups (6, 22, 23). However all 3 studies had significantly lower rates of recurrence than more recent studies, with an average recurrence rate of 11% in both the metronidazole and vancomycin arms. This low rate of recurrence may be due in part to the fact that only one of the studies was done after the emergence of the NAP1 strain, which has been linked to increase rates of recurrence (6). Subsequently, the largest randomized controlled trial on CDI that included vancomycin and metronidazole arms found that vancomycin had lower recurrence rates than metronidazole regardless of disease severity (23 vs. 29%), however this result was not statistically significant (7).

Importantly, both metronidazole and vancomycin alter the intestinal microbiome and thus may predispose to recurrence (26). However, vancomycin is considered a more targeted therapy as its spectrum of activity in the gut is limited predominantly to *Clostridium* and *Enterococcus* species. Metronidazole has a much broader spectrum of activity in the gut and is capable of diminishing most enteric anaerobes, thus when compared to vancomycin, metronidazole could lead to more dysbiosis. This hypothesis has not been documented in previous research, possibly because vancomycin can prolong *C. difficile* carriage. In a study of asymptomatic carriers, vancomycin was shown to reduce *C. difficile* carriage faster than metronidazole, however at the end of the 30 day post-treatment observation period, patients in the vancomycin arm were more likely to still be carriers than patients in the metronidazole arm (27). These data suggest that vancomycin treatment may result in a higher rate of late CDI recurrences than metronidazole. Additionally, even though dual therapy with metronidazole and vancomycin is recommended in

severe complicated CDI, little is known about the rates of recurrence when combination therapy is utilized. Newer targeted therapies, such as fidaxomicin, have shown some promise in reducing recurrence compared to vancomycin, especially in non-NAP1 strains (28, 29). Adjunctive therapies such as rifaximin and probiotics have been utilized, but the effectiveness of these agents is less clear (24). Fecal microbiota transplants, also known as stool transplants, have been shown to be effective in preventing further recurrent CDI in patients who have already had multiple episodes by restoring the healthy intestinal microbiome. However this therapy has not readily been utilized in patients after their initial episode.

Identifying patients who are at risk for recurrence is important for patients to understand their prognosis but also to consider alternative treatment options (such as prolonged therapy, tapering therapy or adjunctive therapy). The causal pathway for recurrent CDI is similar to initial CDI in that both dysbiosis and exposure to *C. difficile* are essential components. Exposure to the bacteria is most often associated with healthcare exposures. Here we evaluate the role of health exposures prior to initial CDI and its association with risk of recurrent disease while controlling for potential confounders. We also assess the risk of recurrent disease associated with initial choice of CDI therapy (vancomycin or metronidazole) as both of these agents, while active against C. difficile, can also contribute to further dysbiosis.

METHODS

Research Goal

This study has two main objectives. The first objective is to estimate the association of the epidemiologic classification of the initial CDI episode with the risk for recurrent CDI. The second objective is to estimate the association of the initial CDI treatment with the risk for recurrent CDI.

Hypotheses

1. Community-associated *C. difficile* infection is associated with a lower risk for recurrent disease than hospital-onset *C. difficile* infection.
2. Initial *C. difficile* infection treated with vancomycin alone is associated with lower risk for recurrent disease than initial *C. difficile* infection treated with metronidazole alone.

Study Design

A prospective, observational cohort study design was utilized to assess factors associated with CDI recurrence within 6 months of initial CDI. The main outcome is recurrent CDI within 2 weeks to 6 months from initial CDI episode. The primary exposure for the first model is epidemiologic classification. The primary exposure for the second model is treatment with vancomycin alone compared to treatment with metronidazole alone.

Patient Selection

The Emerging Infections Program (EIP) is funded by the Centers for Disease Control and Prevention (CDC) and performs active population- and laboratory-based surveillance for bacterial pathogens. Thirty-four counties in ten states (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee) participate in EIP with

approximately 11.2 million persons under surveillance. CDI surveillance was started in 2009 in some states and as late as 2011 in others. Trained surveillance staff at each EIP site performs medical record reviews on positive *C.difficile* tests for residents in the catchment areas. Random stratified sampling is conducted in Colorado and Georgia of all incident CDI tests. In all locations, all community-associated (CA) and community-onset, healthcare facility onset (CO-HCFA) incident cases are reviewed. However, a random 1:10 sample of healthcare facility onset (HCFO) cases is performed at all sites.

An initial CDI case was defined as a patient with their first episode of CDI from January 1, 2013 to December 31, 2013 and who did not have a history of previous positive CDI tests in surveillance. Patients over the age of 17 years were included. Patients had to have documented diarrhea or had received CDI treatment with metronidazole, vancomycin or fidaxomicin for their initial episode to be considered a case.

A recurrent CDI case was defined as a subsequent positive CDI test between 2 and 26 weeks from the initial CDI case. The patient had to have documented diarrhea or had received CDI treatment with metronidazole, vancomycin or fidaxomicin for the recurrent episode in order to be considered a recurrent case. Surveillance was continuous for at least 26 weeks after each initial CDI case identified in 2013.

Cases were excluded from the analysis if the death of the patient was documented after the initial case. Death was ascertained in patient who were hospitalized or in a long term care facility at time of diagnosis and died during that healthcare facility stay. Cases were also excluded if the patient had a recurrent positive test but the recurrent test did not have information available to assess whether the patient had diarrhea or had received CDI treatment.

Variable definitions

The outcome of interest is recurrent CDI 2 weeks to 6 months from the initial CDI case. Two analyses were performed. The primary exposure of interest for the first analysis is

epidemiologic classification (hospital-onset cases compared to community-associated cases). The second analysis was restricted to patients who received either vancomycin alone or metronidazole alone.

Covariates assessed included: age (18-44 years old, 45-64 years old, 65 years or older), sex, race (white, black, other), ethnicity (Hispanic, non-Hispanic, other), documented diarrhea, upper gastrointestinal symptoms (nausea or vomiting), white blood cell count (WBC) (<1 $x10^9$/L or >15 $x10^9$/L), hospitalization (indication and location of discharge), toxic megacolon, ileus, pseudomembranous colitis, colectomy, and intensive care unit (ICU) admission within 2 days of CDI diagnosis. Severe disease was classified as two or more of the following: WBC<$1x10^9$/L or >15 $x10^9$/L, hospitalization due to CDI, toxic megacolon, ileus, pseudomembranous colitis, colectomy, or ICU admission.

Epidemiologic classification was determined as community-associated (CA) if the initial test was collected on an outpatient basis or within 3 days after hospitalization and the patient had no documented overnight stay in a healthcare facility during the previous 12 weeks. Community-onset, healthcare facility associated (CO-HCFA) was designated if the initial test was collected as an outpatient or within 3 days after hospitalization but the patient had documented overnight stay in a healthcare facility during the previous 12 weeks. Healthcare facility onset cases are divided into hospital onset and long term care facility onset. Hospital onset (HO) cases were classified if the initial test was collected after 3 days of hospitalization. Long term care facility onset (LTCFO) cases were identified if the initial test was collected in a long term care facility (including skilled nursing facilities) or the patient was admitted from a long term care facility (LTCF).

Select comorbidities were also assessed including: human immunodeficiency virus (HIV), chronic liver disease, chronic pulmonary disease, chronic renal insufficiency (including hemodialysis use), diabetes mellitus, inflammatory bowel disease (IBD), diverticular disease, peptic ulcer disease (PUD), solid tumors, hematologic malignancy and transplant (stem cell and

solid organ). A modified Charlson score was calculated due to the inability to assess complications and severity of diabetes mellitus and liver failure, respectively, thus all patients with these comorbidities were classified as mild.

Medications given 12 weeks prior to initial CDI were evaluated such as proton pump inhibitors (PPI), H2-blockers, and immunosuppressive therapy (e.g. chemotherapy and steroids). Antibiotics were classified by the class of antibiotic and by the number of different antibiotics the patient received in the 12 weeks prior to initial CDI. CDI treatment was classified as either: metronidazole alone, vancomycin alone, fidaxomicin at any time, or no CDI-specific antibiotic treatment. No CDI treatment referred to patients who did not receive metronidazole, vancomycin or fidaxomicin. For patients who received both metronidazole and vancomycin, but not fidaxomicin, these patients were further classified by whether both metronidazole and vancomycin were utilized concurrently for 2 or more days in a row, which were classified as "concurrent metronidazole and vancomycin." If a patient received both antibiotics, but did not overlap days for 2 or more days, then the patient was classified as "non-concurrent metronidazole and vancomycin." Adjuvant therapy with probiotics, nitazoxanide and rifaxamin were also assessed.

Methods for Assessing Recurrence Risk by Epidemiologic Classification

The first aim of this project is to assess the risk of recurrence by epidemiologic classification. Statistical analyses included descriptive characteristics for all variables. Chi-square and student t-tests was used to assess categorical variables and continuous variables, respectively. Bivariate analyses were performed for categorical variables' association with recurrence by likelihood ratio chi-square test. Variables with $p<0.20$ were included for selection into multivariate logistic regression model.

Previous EIP data suggested that race is often missing from surveillance data. In assessing the risk of recurrence with regards to epidemiologic classification, we developed two

models. The first multivariate logistic regression model utilized a complete case analysis: only including cases where all variables were available (referred to as "complete case model"). The primary exposure of interest was epidemiologic classification (CA, CO-HCFA, HO or LTCFO with CA was the reference group). The outcome was recurrence within 6 months of the initial episode. Covariates were selected by backward selection utilizing stay criteria of 0.05 except age, CDI treatment and epidemiologic classification were forced into the model. Pair-wise interactions were assessed by the Breslow-Day test and were retained in model if $p<0.05$. All reported *p*-values are two sided with α (the significance level<0.05). Odds ratios and 95% confidence intervals were reported.

Multivariate logistic regression model for epidemiologic classification:

Logit P(Recurrence=1)= $\beta_0+\beta_1$(epidemiologic classification)+β_2(treatment)+ β_3(age)+β_4(covariate1)+...

For the second multivariate logistic regression model, multiple imputation by fully conditional specification with discriminant method imputed data for missing variables (referred to as "multiple imputation model"). Imputation was performed based on other known variables, including county, state, and all variables that had $p<0.20$ in bivariate analysis with recurrence. Imputation by these variables ensured an accurate filling of the missing data while holding potential associations between variables intact. The missing data was assumed to be "missing at random," meaning that race data was incomplete due to other factors and not due to race alone. For instance, race was often missing because some healthcare facilities do not record race data, thus race is missing due to the type of healthcare facility. This assumes that people of a certain race are not more or less likely to have missing data compared to other races. Furthermore, missing data was expected to be in an arbitrary pattern rather than a monotone pattern which also influenced the method of imputation. In order to assess the imputed data, we evaluated the

relative increase in variance and the relative efficiency of imputing the data for each variable with missing data. We created five imputed data sets with a burn iteration of 20. Multivariate logistic regression was performed in the same manner as described above for each imputation allowing for parameter estimates for each selected variable by imputation. We then utilized PROC MIANALYZE in SAS version 9.3 (SAS Institute Inc., Cary, NC) in order develop summary parameter estimates for each variable which incorporates the error of each imputation. The final multivariate logistic regression model is the same as listed above.

Methods for Assessing Recurrence Risk by CDI Treatment- Propensity Score

The second aim of this study was to evaluate the risk of recurrence in patients who received vancomycin alone compared to metronidazole alone. We utilized the same data sources, case definitions and variable definitions as described above. We restricted the data to include only patients who received either vancomycin alone or metronidazole alone and who had complete variables for analysis. Therefore we did not utilize imputation for this method.

Because the indication of prescribing metronidazole alone or vancomycin alone may be associated with recurrence, we performed matching by propensity score. Propensity scoring allows for balancing multiple variables while creating a stable model (30). Multivariate logistic regression was performed with the outcome being treatment with vancomycin alone or metronidazole alone. The covariates were chosen based off literature review of variables that are considered to be associated with risk of recurrence. This list included the following 19 variables: age, sex, race, epidemiologic classification, chronic renal insufficiency, diabetes mellitus, inflammatory bowel disease, hematologic malignancy, solid organ transplant, Charlson comorbidity index, proton-pump inhibitor use, immunosuppressive therapy, previous antibiotic use, previous metronidazole use, documented diarrhea, white blood cell count $<1 \times 10^9$/L or $>15 \times 10^9$/L, hospitalization due to CDI, ICU admission and severe disease (as defined above).

Propensity score multivariate logistic regression model:

$$\text{Logit P (treatment=vancomycin)} = \beta_0+\beta_1(\text{age})+\beta_2(\text{sex})....$$

Utilizing the multivariate logistic regression model, predicted probabilities for treatment with vancomycin was evaluated. Discrimination of the propensity score was evaluated by the area under the receiver operator curve, or c-statistic. Calibration of the propensity score was evaluated by the Hosmer and Lemeshow Goodness of Fit test. Patients were then stratified by propensity score decile. Chi-square test was utilized to assess the categorical variables by treatment with vancomycin or metronidazole. All reported *p*-values are two sided with α (the significance level<0.05). Balance diagnostics were further evaluated by assessing the standardized difference of each variable between the vancomycin group and the metronidazole group.

Subsequently, the final multivariate logistic regression model was developed. The outcome for this model was recurrence 2 weeks to 6 months of the initial CDI episode. The primary exposure was treatment with vancomycin alone or metronidazole alone. The covariate was propensity score decile. The final estimates were assessed in odds ratios with 95% confidence intervals with the reference group being metronidazole alone.

Final Multivariate logistic regression model for treatment:

$$\text{Logit P(Recur=1)}= \beta_0+\beta_1(\text{treatment})+\beta_2(\text{propensity decile})$$

Sample size calculation for propensity score model

Based on preliminary data from a previous clinical trial, the recurrence rate in the metronidazole arm was 29% whereas in the vancomycin arm was 23% (25). Previous Georgia EIP data showed that the ratio of patients treated with metronidazole compared to those treated with vancomycin was 3 to 1. In order to obtain an alpha of 0.05 and a power of 80%, we calculated that 580 patients would need to be in the vancomycin arm and 1,740 patients would need to be in the metronidazole arm for a total sample of 2,320.

Database management

Data was entered into a Microsoft Access 2007 database (Microsoft Corp., Redmond, WA) and statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

IRB Approval

This analysis is part of the population-based *Clostridium difficile* infection surveillance study which has been approved by the institutional review boards at the Centers for Disease Control and Prevention and at each participating EIP sites including Emory University and Georgia Department of Public Health.

RESULTS

Initial cases

In 2013, 13,723 positive *C. difficile* tests were identified in patients who did not have a previous positive test under surveillance. Patient selection is described in Figure 1. The number of initial cases with documented diarrhea or CDI treatment was 5,161. A total of 4,790 were included in the analysis; 128 initial cases died and 243 initial cases had a recurrent positive test but clinical information regarding diarrhea or subsequent treatment was unavailable. Seventeen percent of initial cases (843 cases) had a recurrent CDI episode 2 to 26 weeks from the initial case. Univariate and bivariate analyses of patient and clinical characteristics associated with recurrence are described in Tables 1-3. Of the 4,790 initial cases, 42% were over age 65 years, 62% were female and 63% were community-associated, 28% community-onset healthcare facility associated, 6% hospital onset and 4% long term care facility onset. Of the cases with available race information (n=3,630, 76% of all initial cases), 82% were white and 15% were black. Comorbid conditions were common with 57% of cases having a Charlson score of one or more. Similarly, preceding antibiotic use was seen in 71% of initial cases; cephalosporins and fluoroquinolones being the most common (34% each) followed by beta-lactam/beta-lactamase inhibitor antibiotics (24%). Eight percent of initial cases had severe disease. Sixty-three percent were treated with metronidazole alone, 14% with vancomycin alone, 9% with concurrent metronidazole and vancomycin, 10% with non-concurrent metronidazole and vancomycin, 1% with fidaxomicin, and 3 % has no CDI-specific antibiotic treatment. Probiotics were utilized in 17% of initial cases.

Bivariate analyses with risk of recurrence

Compared to community-associated (CA) CDI, hospital-onset (HO) CDI was not associated with risk of recurrence (OR 1.04 95%CI 0.76-1.43). However community-onset healthcare facility-associated (CO-HCFA) cases and long term care facility-onset (LTCFO) cases

were associated with higher risk of recurrence compared to CA cases (OR 1.32 95%CI 1.12-1.56 and OR 1.88 95%CI 1.32-2.68, respectively).

Age over 65 years, white race, chronic pulmonary disease, chronic renal insufficiency (with or without hemodialysis), diabetes mellitus, Charlson score of one or more, and PPI use or immunosuppressive use in the preceding 12 weeks were all associated with increased risk of recurrence ($p<0.05$). HIV infection was associated with a lower risk of recurrence (OR 0.39 95%CI 0.16-0.98). Antibiotic use in the 12 weeks prior to CDI was associated with recurrence ($p<0.01$). Prior cephalosporin use was associated with higher risk of recurrence compared to use of other antibiotic classes (OR 1.21 95%CI 1.01-1.45), a finding not demonstrated with other antibiotic classes, including beta-lactam/beta-lactamase inhibitors, carbapenems, fluoroquinolones and clindamycin ($p>0.05$). Severe CDI was associated with increased risk of recurrence (OR 1.36 95%CI 1.06-1.77), but abnormal white blood cell count ($<1 \times 10^9$/L or $>15 \times 10^9$/L) alone was not associated with recurrence ($p=0.93$).

In terms of treatment, vancomycin alone and metronidazole alone had similar rates of recurrence (OR 1.23 95%CI 0.98-1.53). Compared to metronidazole alone, treatment with both metronidazole and vancomycin (concurrently and non-concurrently) and fidaxomicin was associated with higher risk of recurrence ($p<0.05$). Treatment with no CDI-specific antibiotic was associated with a lower risk of recurrence (OR 0.53 95%CI 0.29-0.98). Probiotic use had no effect on recurrence risk ($p=0.27$).

Multivariate logistic regression- Epidemiologic classification

The same variables were included in the complete case model and the multiple imputation model (Appendix 2). Covariates included age, initial CDI treatment, sex, race, chronic renal insufficiency, diabetes mellitus, number of preceding antibiotics, and reason for admission. In both the complete case and the multiple imputation models, epidemiologic classification was

not significantly associated with recurrence (for HO vs. CA p=0.68; for LTCFO vs. CA p=0.15; for CO-HCFA vs. CA p=0.51).

In the multiple imputation model, there was no difference in recurrence between metronidazole alone and vancomycin alone (p=0.20). As shown in Table 4, however, treatment with both vancomycin and metronidazole, concurrently or non-concurrently, was associated with increased risk of recurrence (p<0.01). Treatment with fidaxomicin also had increased risk (adjusted OR 2.55 95%CI 1.31-4.95) whereas use of no CDI-specific antibiotic was associated with lower risk of recurrence (adjusted OR 0.54 95%CI 0.29-1.00). Age over 65 years, female sex, white race compared to black race and other race, chronic renal insufficiency with hemodialysis use, diabetes mellitus, preceding antibiotic use were associated with increased risk of recurrence (p<0.05). Hospitalization for a reason other than CDI was associated with a lower risk of recurrence (adjusted OR 0.72 95%CI 0.59-0.89).

In the complete case model, most parameter estimates were similar to the multiple imputation model. However no statistical association with recurrence risk was noted for age over 65, female sex, no CDI treatment, and non-white, non-black race in the complete case model(p>0.05).

Propensity score model

Of the initial cases, 2,861 were treated with metronidazole alone and 626 were treated with vancomycin alone. The distribution of patient and clinical characteristics are described in Table 5. By adjusting for the 19 covariates listed in the methods, the propensity score model had fair discrimination between patients treated with metronidazole alone compared to vancomycin alone (c-statistic=0.98) as well as appropriate calibration by Hosmer and Lemeshow goodness of fit test (p=0.43) (Figures 2-3). Patients were stratified by propensity score decile. For each decile group, the number of vancomycin patients ranged from 18-90 and the number of metronidazole patients ranged from 123-205 (Figure 4). The balance of covariates before and after propensity

score stratification is shown in Table 5. Balance diagnostics is further assessed by assessing the absolute standardized difference before and after propensity score stratification (Figure 5). The final logistic regression model adjusting for propensity score decile showed that vancomycin alone and metronidazole alone had the same risk of recurrence (adjusted OR 1.23 95% CI 0.93-1.63) (Table 6).

DISCUSSION

Recurrent *Clostridium difficile* infections continue to be common with at least 17% of the patients in the study developing a recurrent episode within six months of the initial case. Previous studies have utilized varying definitions for recurrent disease, including different follow up periods and requirements for signs and symptoms of active disease. National guidelines have recommended utilizing a definition of recurrence as a positive test two to eight weeks after the initial test (4). This definition is most helpful in understanding transmission events as recurrent episodes within 8 weeks of the initial CDI episode are more likely to be caused by the same strain of *C. difficile* (relapse) compared to those after 8 weeks, which are more likely to be due to acquisition of a new strain of *C. difficile* (reinfection). Differentiating between relapse and reinfection is important from a public health/infection prevention perspective, where much effort is placed on preventing transmission events. However, from an individual patient's perspective and a clinician's perspective, the difference between relapse and reinfection is not clinically meaningful as the presentation and approaches to treatment are generally the same. Therefore, following patients for 6 months from initial case allows for a better assessment of the individual patient burden of recurrent disease while assessing risk factors for relapse and reinfection simultaneously.

Potential interventions to prevent recurrences are being developed, such as vaccines and monoclonal antibodies (31, 32). In this setting understanding who is at highest risk for recurrence is important in developing clinical trials to assess the efficacy of those interventions. This study showed that the epidemiologic classification was not associated with risk of recurrence. Importantly, hospital-onset (HO) disease and community-associated (CA) disease had the same risk of recurrent CDI (p=0.68). Although not statistically significant, there is a suggestion that long term care facility-onset (LTCFO) cases may have a higher risk of recurrence than CA cases (adjusted OR 1.31 95%CI 0.90-1.91). The random sampling of healthcare facility-onset cases limited the number of LTCFO cases, which in turn limited the power of this analysis. These are

important findings as potential interventions (such as vaccines and monoclonal antibodies) may be assessed first in hospitalized patients, therefore a better understanding of which hospitalized patients are at greatest risk is important. Residents of LTCFs may warrant further study as these patients are at high risk of continued dysbiosis (e.g. continued antibiotic exposure), have poor immune response (e.g. older age) and are in environments conducive to continued *C. difficile* exposure (e.g. healthcare settings). Therefore long term care facility residents have multiple reasons to be at increased risk for relapse and reinfection.

This study demonstrated no difference in CDI recurrence between patients treated with vancomycin alone and metronidazole alone for the initial episode by multivariate logistic regression and by propensity score stratification ($p>0.05$ in all models). This finding is consistent with the largest randomized controlled trial on *C. difficile* that was recently published (7). In the subgroup analysis, Johnson et. al. showed that for initial CDI there was no difference in recurrence in patients treated with vancomycin or metronidazole (19% in both groups). However for patients with recurrent disease, there was a trend that vancomycin may be associated with lower recurrences than metronidazole (25% vs. 36% $p=0.08$) (7).

We also found that treatment that includes both vancomycin and metronidazole or treatment with fidaxomicin were associated with higher risk of recurrence than use of metronidazole alone ($p<0.05$). Although this result is adjusted for multiple factors such as age, sex, race, CRI, DM, preceding antibiotic use and hospitalization, there is likely confounding by indication in this result. CDI treatment guidelines recommend the use of both vancomycin and metronidazole concurrently for severe disease. Severe disease is defined as an elevated white blood cell count or acute renal insufficiency; however, clinicians may take other factors into account in their decision to utilize this combination. Similarly, in 2013 (at the time of this study), fidaxomicin was a newly approved antibiotic for CDI treatment and use was limited and it was likely utilized in patients with the most complicated initial episodes. The multiple imputation model showed that patients who received no CDI-specific antibiotic had lower risk of recurrence, which supports earlier

studies that suggested that stopping the microbiome-offending agent (such as other antibiotics) may be a sufficient intervention in select patient populations, but they may also represent patients with the mildest disease that did not warrant specific CDI treatment intervention.

This study is one of the largest collections of initial CDI cases in the United States and it includes a diverse geographical distribution of cases, which improves the generalizability of our findings. Utilizing ongoing population-based surveillance, we were able to ensure that that the initial cases did not have a previous episode in at least the prior two years. Furthermore by restricting our analysis to cases that had symptoms or treatment strengthened the assumption that these cases represented true infection rather than ongoing colonization. This population-based network also allowed the inclusion of outpatient initial CDI cases, but, perhaps more importantly, also included outpatient recurrent cases. This structure is important as 34% of recurrent CDI cases were diagnosed at a different laboratory than the initial case.

The results regarding CDI treatment are limited by potential unknown confounders, particularly confounding by indication as previously mentioned. Furthermore the definitions of vancomycin alone and metronidazole alone groups may limit the generalizability of these results as patients who switched from one medication to the other were analyzed separately. Furthermore, the propensity score stratification model may have benefited from improved balance of the variables with further high-dimensional manipulation of the variables through exponentiation of variables and including multi-level interactions into the model. In assessing for outcomes for CDI, we were unable to assess whether patients achieved clinical cure at the end of treatment, thus we were unable to clearly assess whether the recurrent episode was a continuation of the initial case or truly a new case of CDI.

In conclusion, we have shown that hospital-onset CDI and community-associated CDI have similar risk of recurrence. Further study is indicated to better understand the risk of recurrence in long term care facility-onset cases. For initial cases, we saw no difference in recurrence risk with treatment with vancomycin alone compared to metronidazole alone. Further

study is warranted in understanding the effect of treatment on patients being treated for a first recurrence. Understanding the risk factors for recurrent disease is especially important in the setting of potential interventions being developed to prevent recurrence. Developing adequate prediction tools for recurrence will be important in developing those clinical trials and estimating the impact of those interventions on the burden of *C. difficile* infection.

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TABLES

Table 1. Description of initial cases and bivariate analysis of variables association with recurrent *C. difficile* infection (CDI) - Patient factors. CA: community associated; CO-HCFA: community onset-healthcare facility associated; HCFO: healthcare facility onset; HO: hospital onset; LTCFO: long term care facility onset

Characteristic of Initial CDI	All initial CDI N (% of total)	Patients with Recurrence N (% of group)	OR	95% CI	Global p-value[1]
Age (years)					<0.01*
18-44	1165 (24)	161 (14)	Ref		
46-64	1620 (34)	276 (17)	1.28	1.04-1.58	
65+	2005 (42)	406 (20)	1.58	1.30-1.93	
Sex					0.05*
Male	1843 (38)	299 (16)	Ref		
Female	2947 (62)	544 (18)	1.17	1.00-1.37	
Race (n=3,630)					<0.01*
White	2964 (82)	564 (19)	Ref		
Black	531 (15)	76 (14)	0.71	0.55-0.92	
Other	135 (4)	18 (13)	0.66	0.40-1.09	
Ethnicity (n=3,259)					0.55
Not Hispanic/Latino	2929 (90)	545 (19)	Ref		
Hispanic/Latino	330 (10)	57 (17)	0.91	0.68-1.23	
Epidemiologic classification of initial case					<0.01*
CA	2991 (63)	480 (16)	Ref		
CO-HCFA	1328 (28)	268 (20)	1.32	1.12-1.56	
HCFO (HO+LTCFO)	471	95 (20)			
HO	301 (6)	50 (17)	1.04	0.76-1.43	
LTCFO	170 (4)	45 (26)	1.88	1.32-2.68	
Diarrhea (n=4710)					0.05*
No diarrhea	412 (9)	58 (14)	Ref		
Documented diarrhea	4298 (91)	766 (18)	1.32	0.99-1.77	
Nausea and/or vomiting (n=4522)					0.36
No nausea/vomiting	2889 (64)	516 (18)	Ref		
Nausea/vomiting	1633 (36)	274 (17)	0.93	0.79-1.09	

[1]: likelihood ratio chi square

*: p<0.20 selected for multivariate analyses

Table 2. Description of initial cases and bivariate analysis of variables association with recurrent *C. difficile* infection (CDI) - Comorbidities and preceding medications. HIV: human immunodeficiency virus; IBD: inflammatory bowel disease; PPI: proton pump inhibitors.

Characteristic of Initial CDI	All initial CDI N (% of total)	Patients with Recurrence N (% of group)	OR	95% CI	Global p-value[1]
Select Comorbidities			Ref=not having condition		
HIV +	64 (1)	5 (8)	0.39	0.16-0.98	0.02*
Liver disease	181 (4)	28 (18)	0.85	0.57-1.28	0.44
Pulmonary disease	796 (17)	161 (20)	1.23	1.02-1.49	0.04*
Renal insufficiency					<0.01*
No hemodialysis	464 (10)	105 (23)	1.47	1.17-1.86	
Hemodialysis	171 (4)	50 (29)	2.08	1.48-2.92	
Diabetes mellitus	1033 (22)	229 (22)	1.46	1.23-1.73	<0.01*
IBD	297 (6)	52 (18)	0.99	0.73-1.35	0.97
Diverticular disease	463 (10)	88 (19)	1.11	0.87-1.42	0.41
Peptic ulcer disease	118 (2)	24 (20)	1.20	0.76-1.89	0.44
Solid tumor	403 (8)	78 (19)	1.14	0.88-1.47	0.34
Hematologic malignancy	148 (3)	35 (24)	1.47	1.00-2.17	0.06*
Stem cell transplant	18 (0.4)	5 (28)	1.81	0.64-5.08	0.29
Solid organ transplant	74 (2)	14 (19)	1.09	0.61-1.97	0.77
Charlson score					<0.01*
0	2076 (43)	304 (15)	Ref		
1-2	1583 (33)	286 (18)	1.29	1.08-1.53	
3+	253 (24)	253 (22)	1.68	1.40-2.02	
Pregnant or post-partum (n=2935)	40 (1)	5 (13)	0.63	0.25-1.61	0.30
Medications 12 weeks prior to CDI			Ref= not received class of med		
PPI (n=4642)	1816 (39)	351 (19)	1.18	1.02-1.38	0.03*
H2 blockers (n=4629)	532 (11)	89 (17)	0.97	0.75-1.26	0.47
Immunosuppressive therapy (n=4619)	1055 (23)	211 (20)	1.20	1.01-1.43	0.04*
Number of different antibiotics used 12 weeks prior to CDI (n=4629)					<0.01*
0	1379 (30)	182 (13)	Ref		
1	1606 (35)	306 (19)	1.55	1.27-1.89	
2 or more	1644 (36)	339 (21)	1.71	1.40-2.08	
Class of antibiotic received 12 weeks prior to CDI (n=3250)			Ref= received antibiotic not in same class		
B-lactam/B-lactamase inhibitors	777 (24)	159 (20)	1.05	0.86-1.29	0.62
Cephalosporins	1101 (34)	241 (22)	1.21	1.01-1.45	0.04*
Carbapenems	169 (5)	31 (18)	0.90	0.61-1.35	0.61
Other B-lactam	283 (9)	52 (18)	0.90	0.65-1.23	0.51
Fluoroquinolones	1100 (34)	220 (20)	1.02	0.85-1.22	0.88
Clindamycin	442 (14)	91 (21)	1.06	0.82-1.35	0.68
Other antibiotic	1471 (45)	295 (20)	1.02	0.86-1.22	0.79

[1]: likelihood ratio chi square

*: p<0.20 selected for multivariate analyses

Table 3. Description of initial cases and bivariate analysis of variables association with recurrent *C. difficile* infection (CDI) - Severity and treatment. Severe disease: Two or more of the following: white blood cell count <1,000 or >15,000, hospitalized for CDI, toxic megacolon, ileus, pseudomembranous colitis, colectomy, or ICU admission)

Characteristic of Initial CDI	All initial CDI N (% of total)	Patients with Recurrence N (% of group)	OR	95% CI	Global p-value[1]
Disease Severity			Ref=not having clinical finding		
WBC<1000 or >15,000	797 (19)	145 (18)	0.99	0.81-1.21	0.93
Hospitalization					0.05*
Not hospitalized	2542 (53)	448 (18)	ref		
Admit for CDI	952 (20)	189 (20)	1.16	0.96-1.40	
Admit for other	1285 (27)	204 (16)	0.88	0.74-1.06	
Toxic megacolon, ileus, and/or pseudomembranous colitis	183 (4)	29 (16)	0.91	0.61-1.37	0.66
Colectomy	11 (0.2)	1 (9)	0.47	0.06-3.68	0.43
ICU admission	104 (2)	15 (14)	0.78	0.45-1.36	0.38
Severe disease[5]	371 (8)	82 (22)	1.36	1.06-1.77	0.02*
Initial CDI treatment (n=4529)					<0.01*
Metronidazole alone	2861 (63)	464 (16)	ref		
Vancomycin alone	626 (14)	120 (19)	1.23	0.98-1.53	
Both Metronidazole and vancomycin					
Concurrently for ≥2 days	426 (9)	95 (22)	1.48	1.16-1.90	
Not concurrent	449 (10)	103 (23)	1.54	1.21-1.96	
Fidaxomicin at anytime	39 (1)	14 (36)	2.89	1.49-5.61	
No CDI-specific antibiotic	128 (3)	12 (9)	0.53	0.29-0.98	
Concurrent CDI treatment			Ref=not taking medication		
Probiotics	814 (17)	157 (19)	1.12	0.92-1.36	0.27
Rifaxamin	18 (0.4)	3 (17)	0.92	0.27-3.19	0.89
Nitazoxanide	15 (0.3)	2 (8)	0.71	0.16-3.14	0.64

Table 4. Multivariate logistic regression model assessing association of epidemiologic classification and recurrence utilizing multiple imputations for missing data. CA: community associated; CO-HCFA: community onset-healthcare facility associated; HO: hospital onset; LTCFO: long term care facility onset

Variable	Adjusted OR	95% CI	p-value
Epidemiologic classification			
CA	ref		
CO-HCFA	1.07	0.88-1.28	0.51
HO	0.93	0.64-1.33	0.68
LTCFO	1.31	0.90-1.91	0.15
Initial CDI treatment			
Metronidazole alone	ref		
Vancomycin alone	1.16	0.92-1.47	0.20
Both vancomycin and metronidazole			
Concurrent	1.49	1.14-1.95	<0.01*
Switch	1.52	1.18-1.95	<0.01*
Fidaxomicin	2.55	1.31-4.95	<0.01*
No treatment	0.54	0.29-1.00	0.05*
Age (years)			
18-44	Ref		
45-64	1.20	0.96-1.48	0.11
65+	1.36	1.10-1.69	<0.01*
Sex			
Female compared to male	1.23	1.05-1.44	0.01*
Race			
White	ref		
Black	0.70	0.54-0.92	0.01*
Other	0.60	0.38-0.96	0.03*
Chronic renal insufficiency			
No CRI	ref		
CRI without hemodialysis	1.25	0.98-1.60	0.08
CRI with hemodialysis	2.18	1.51-3.15	<0.01*
Diabetes mellitus	1.33	1.11-1.60	<0.01*
Number of preceding antibiotics			
None	Ref		
1	1.48	1.20-1.82	<0.01*
2 or more	1.51	1.22-1.88	<0.01*
Hospitalization			
Not hospitalized	Ref		
Hospitalized, admission for CDI	0.87	0.71-1.08	0.20
Hospitalized, admission not for CDI	0.72	0.59-0.89	<0.01*

*: p-value<0.05

Table 5. Balance of patient characteristics between patients treated with metronidazole compared to vancomycin before and after propensity score (PS) adjustment

Characteristic of Initial CDI	Treated with metronidazole N (%)	Treated with vancomycin N (%)	p-value	
			Before PS-adjustment	After PS-adjustment
Age (years) (n=3487)	n=2861	n=626	<0.01	0.88
18-44	742 (26)	125 (20)		
46-64	986 (34)	192 (31)		
65+	1133 (40)	309 (49)		
Female	1754 (61)	385 (62)	0.93	0.96
Race (n=2,610)	n=2119	n=491	<0.01	0.98
White	1704 (80)	411 (84)		
Black	334 (16)	51 (10)		
Other	81 (4)	29 (6)		
Epidemiologic classification of initial case (n=2610)	n=2861	n=626	<0.01	0.999
CA	1905 (67)	351 (56)		
CO-HCFA	704 (25)	187 (30)		
HO	155 (5)	56 (9)		
LTCFO	97 (3)	32 (5)		
Select Comorbidities (n=3487)	N=2861	N=626		
Chronic renal insufficiency			<0.01	0.98
CRI not hemodialysis	241 (8)	83 (13)		
Hemodialysis prior to CDI	86 (3)	28 (4)		
Diabetes mellitus	583 (20)	146 (23)	0.10	0.96
Inflammatory bowel disease	143 (5)	63 (10)	<0.01	0.64
Hematologic malignancy	78 (3)	27 (4)	0.04	0.94
Solid organ transplant	38 (1)	16 (3)	0.04	0.86
Charlson score (n=3487)			<0.01	0.95
0	1318 (46)	241 (38)		
1-2	930 (32)	209 (33)		
3+	613 (21)	176 (28)		
Medications 12 weeks prior to CDI				
PPI (n=3390)	1000 (36)	369 (44)	<0.01	0.97
Immunosuppressive therapy (n=3375)	551 (20)	187 (31)	<0.01	0.92
Number of different antibiotics used 12 weeks prior to CDI (n=3384)	N=2769	N=615	<0.01	0.996
0	875 (32)	154 (25)		
1	971 (35)	182 (30)		
2 or more	923 (33)	279 (45)		
Prior metronidazole use	245 (9)	104 (17)	<0.01	0.78
Documented Diarrhea (n=3413)	2539 (91)	541 (88)	0.07	0.97
Disease Severity				
WBC<1000 or >15,000 (n=3005)	317 (13)	141 (16)	<0.01	0.78
Hospitalization (n=3480)			<0.01	0.92
Not hospitalized	1737 (61)	281 (45)		
CDI primary reason	428 (15)	142 (23)		
CDI not primary reason	689 (24)	203 (32)		
ICU admission (n=3466)	28 (1)	16 (3)	<0.01	0.69
Severe disease	125 (4)	65 (10)	<0.01	0.64

Table 6. Multivariate logistic regression model assessing *C. difficile* initial treatment with vancomycin alone compared to metronidazole alone adjusting for propensity to receive vancomycin.

Variable	OR Vancomycin compared to Metronidazole	95% CI	p-value
Crude OR (n=3487)	1.23	0.98-1.53	0.07
OR adjusted for PS decile (n=2132)	1.23	0.93-1.63	0.15
OR adjusted for PS linear (n=2132)	1.24	0.94-1.64	0.13

FIGURES

Figure 1. Patient selection. Of the 13,723 initial positive tests assessed, 4,790 adults were included in final analysis. CA: community associated; CO-HCFA: community onset-healthcare facility associated; HCFO: healthcare facility onset

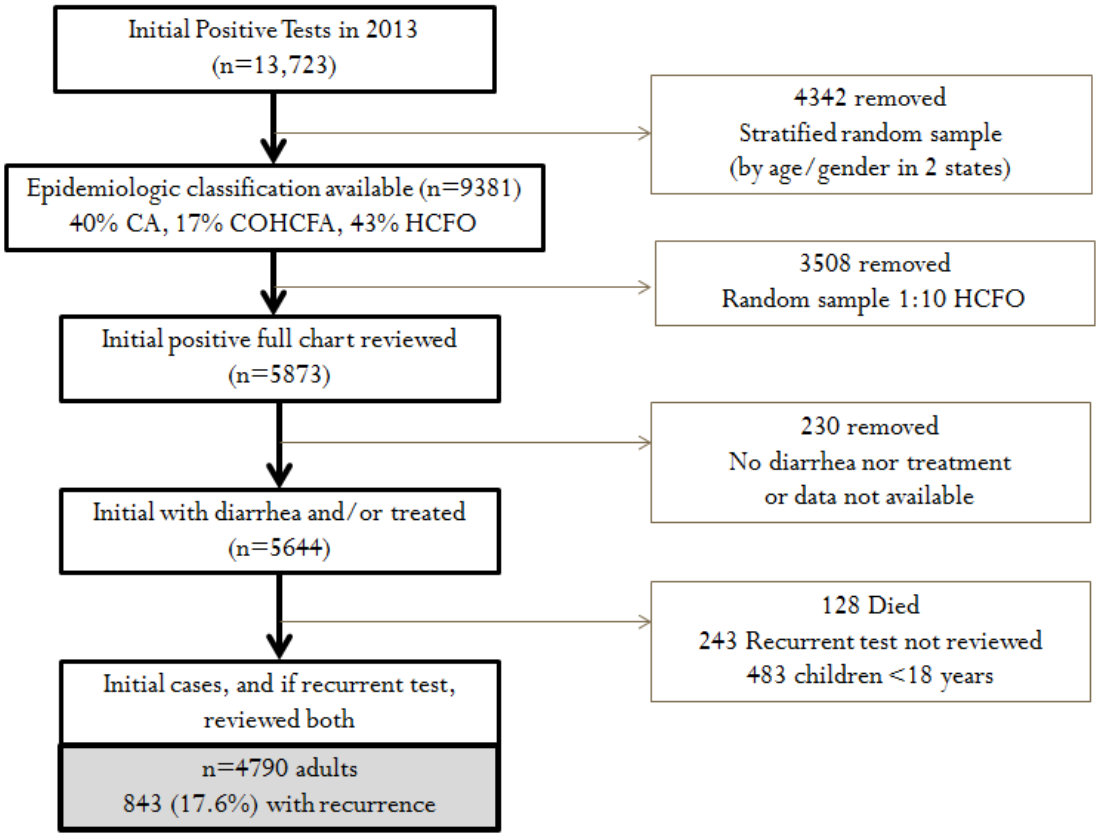


Figure 2. Receiver operator curve predicting Vancomycin use compared to metronidazole use for treatment of initial CDI. C-statistic=0.68.

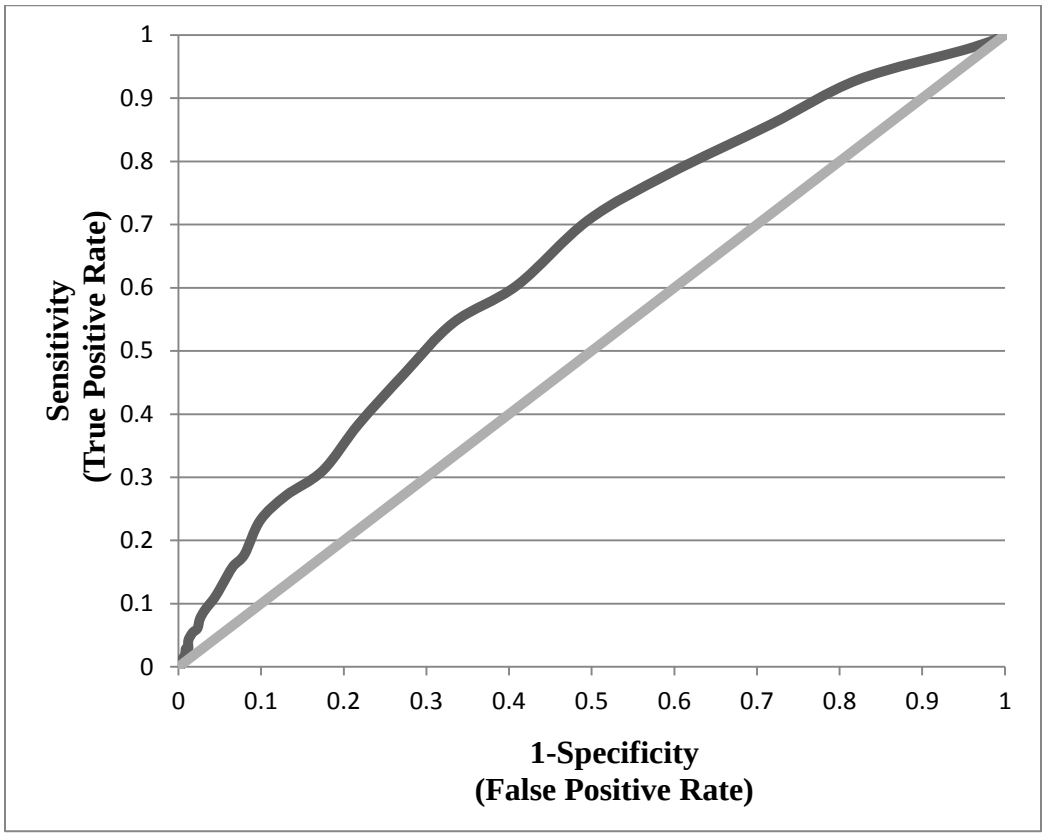


Figure 3. Distribution of predicted probabilities of receiving vancomycin in patients observed to have received metronidazole compared to those who received vancomycin. Diamond: median. Error bars denote range. Dark gray box denotes 50-75 percentile. Light gray box denotes 25-50 percentile.

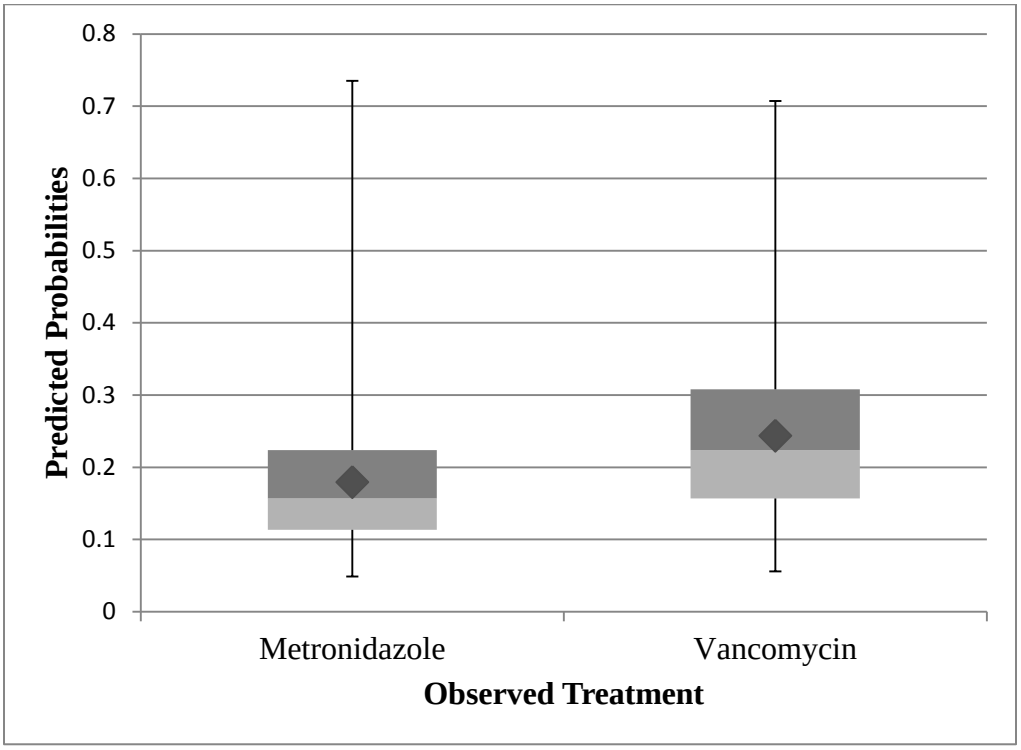


Figure 4. Number of initial cases treated with metronidazole and vancomycin by propensity score decile stratification.

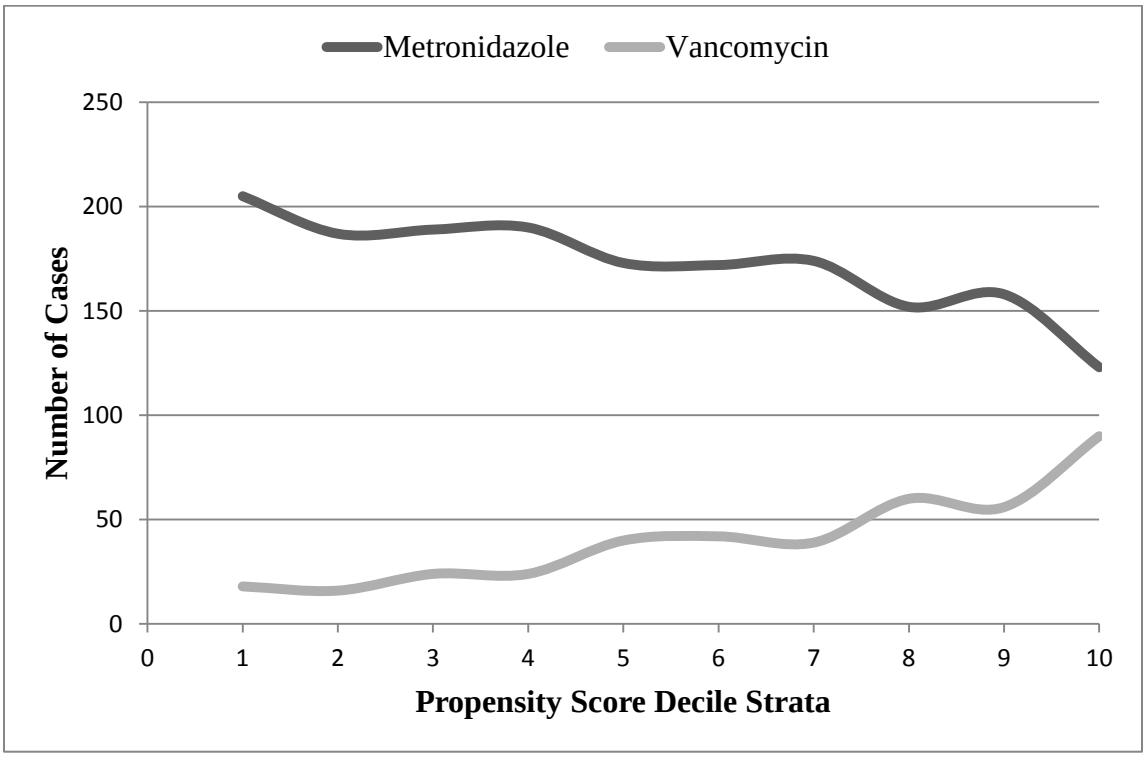
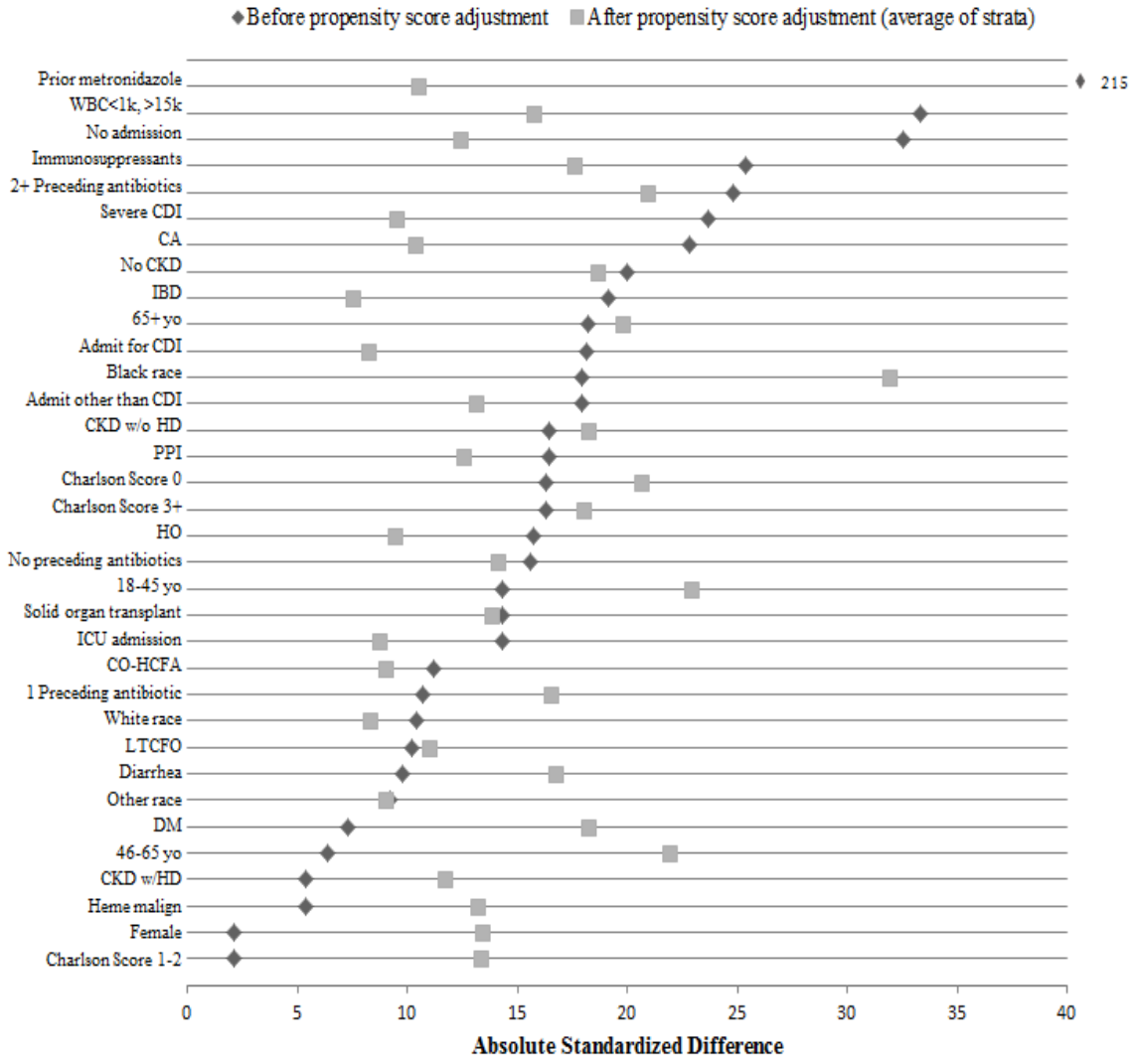
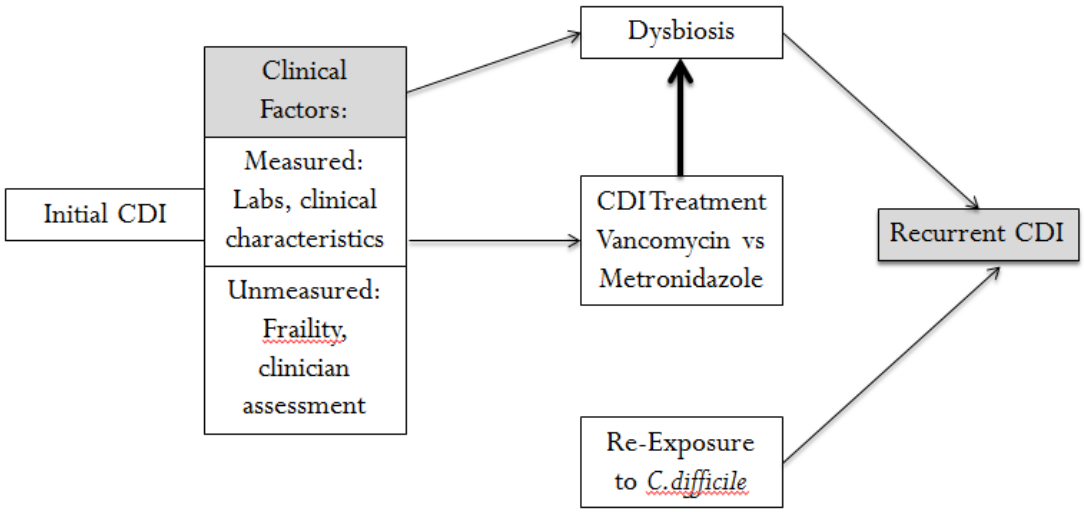


Figure 5. Absolute standardized difference before and after propensity score stratification by decile. Variables with an absolute standardized difference less than ten are considered to be in balance between groups (CITATION). WBC: white blood cell count <1 x10^9/L or >15 x10^9/L; CDI: *C. difficile* infection; CA: community associated; CKD: chronic kidney disease; IBD: inflammatory bowel disease; HD: hemodialysis; PPI: proton pump inhibitor use; HO: hospital onset; ICU: intensive care unit; CO-HCFA: community onset-healthcare facility associated; LTCFO: long term care facility onset; DM: diabetes mellitus.



APPENDICES

Appendix 1. Causal diagram for factors influencing recurrent *C. difficile* infection



Appendix 2: Final Multivariate logistic models

Multivariate logistic regression model assessing the association of epidemiologic classification and recurrence:

Logit P(Recurrence=1)= β_0+β_1(epidemiologic classification)+ β_2(treatment) + β_3(age)+β_4(sex)+β_5(race)+ β_6(chronic renal insufficiency)+ β_7(diabetes mellitus)+ β_8(preceding antibiotics) + β_9(hospitalization)

Propensity score model assessing the association of CDI treatment and recurrence

Propensity score multivariate logistic regression model:

Logit P(treatment=vancomycin)= β0+β1(age)+β2(sex) + β3(epidemiologic classification)+β4(intensive care unit admission)+β5(race)+ β6(chronic renal insufficiency)+ β7(diabetes mellitus)+ β8(severe disease) + β9(inflammatory bowel disease)+ β10(hematologic malignancy)+β11(solid organ transplant)+β12(Charlson score)+ β13(proton pump inhibitor use)+ β14(immunosuppressive use)+ β15(preceding antibiotics) + β16(prior metronidazole use) + β17(diarrhea)+β18(white blood count) +β19(hospitalization)

Final multivariate logistic regression model for treatment:

Logit P(Recur=1)= β_0+β_1(treatment)+β_2(propensity decile)

Appendix 3. Relative efficiency of final multiple imputation model after imputing missing data for selected variables. N=4790.

Variable	Number missing	Relative Increase in Variance	Fraction Missing Information	Relative Efficiency
Race: black vs. white	1160	0.14	0.13	0.974
Race: other vs. white		0.02	0.02	0.997
Number of Preceding Antibiotics (1 vs. 0)	161	0.05	0.05	0.990
Number of Preceding Antibiotics (2+ vs. 0)		0.03	0.02	0.995
Hospitalized for CDI vs. Not hospitalized	11	0.01	<0.01	0.998
Hospitalized not for CDI vs. Not hospitalized		<0.01	<0.01	>0.999
No treatment vs. Metronidazole	261	0.09	0.08	0.983
Vancomycin vs. Metronidazole		0.06	0.06	0.988
Fidaxomicin vs. Metronidazole		0.07	0.06	0.987
Concurrent vs. Metronidazole		0.03	0.03	0.993
Nonconcurrent vs. Metronidazole		0.02	0.02	0.995

Appendix 4. Propensity score stratum specific odds ratio for recurrence (vancomycin compared to metronidazole) compared to adjusted propensity score model and unadjusted model.