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Characteristics associated with short-term risk of
C. difficile infection among hospitalized patients

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Characteristics and antibiotic use associated with short-term risk of *C. difficile* infection among hospitalized patients

By

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B.A., Taylor University, 1998
M.D. Indiana University, 2002

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An abstract of
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Abstract

Characteristics and antibiotic use associated with short-term risk of *C. difficile* infection among hospitalized patients

By Colleen Suzanne Kraft

Clostridium difficile infection (CDI) is the most common cause of infectious healthcare-associated diarrhea. Factors associated with the short-term risk of CDI have not been evaluated. This case-control study examined patient characteristics associated with short-term risk of CDI within 14 days among hospitalized patients with multiple tests who initially test negative. Patients were defined as cases if they had initially tested negative by polymerase chain reaction (PCR) followed by a positive PCR test within 14 days. Controls were drawn from a population of patients from the same time period who had repeat testing and no positive result within 14 days of an initial negative PCR test. Each case was matched with three controls by age and days of hospitalization prior to first PCR test. Conditional logistic regression was used to assess the association between patient characteristics and antibiotic classes and short-term risk of *C. difficile*. Of 750 patients who had a test repeated within 14 days, 30 acquired *C. difficile*. There was a trend for patients with recent gastrointestinal procedure (odds ratio [OR] 2.41, 95% confidence interval [CI] 0.84, 6.88) for short-term acquisition of CDI. Cases had a higher proportion of recent intravenous vancomycin use within 8 weeks prior to first PCR test (OR 3.38, 95% CI 1.34, 8.49). Controls had a higher proportion of recent antiviral use (OR 0.30, 95% CI 0.11, 0.83) as compared to cases. Only 4.0% (30/750) of this study population had short-term acquisition of *C. difficile* and 1.3% (10/750) of the short-term cases of CDI were detected within 7 days. The association with previous intravenous vancomycin use previously has not been described in patients with short-term CDI. The practical implications for this in terms of repeated testing may include eliciting this antibiotic history when clinicians request testing earlier than 7 days in a hospitalized patient.

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INTRODUCTION

Clostridium difficile is the leading cause of antibiotic-associated diarrhea, and it is most often healthcare related in origin (1). The healthcare setting provides environmental source for the spores of the bacterium, as well as the antibiotic pressure that allows the organism to overgrow in the colon and cause clinical disease (1, 2). Clinical microbiological testing for *C. difficile* organism has changed dramatically in the last few years from the use of an insensitive enzyme immunoassay (EIA) for *C. difficile* toxins A and/or B to the use of real-time polymerase chain reaction (PCR) that amplifies the *tcdB* gene in the pathogenicity locus of the bacterium (3, 4). Given the high sensitivity of PCR testing and the low incidence of short-term acquisition (<14 days) of *C. difficile* infection (CDI), most institutions recommend against repeat testing within 7 days because there is "no increase in diagnostic yield" was reported in two small studies (5, 6). In these two studies, only 2.1-3.4% of PCR tests were positive for *C. difficile* within 14 days of an initially negative PCR test. If you consider the individual rather than the overall percentage of patients who have short-term acquisition, repeated testing would have been helpful for that individual in order to have prompt isolation of index cases and for the overall management of this infection (4). On the one hand, laboratories are looking to automate the ability to dissuade physicians from ordering this test again within 7 days (7). On the other hand, the consideration of infection prevention and rapid diagnosis in an individual patient needs to be balanced against the cost of testing many individuals unnecessarily. Patients with repeat testing are exclusively hospitalized patients in these studies, and the patient factors associated with CDI acquisition were age, male sex, inpatient location, ongoing antibiotic treatment, immunosuppression, malignancy, current

hospitalization and recent gastrointestinal (GI) procedure (8-11). However, these factors are not specific to short-term acquisition of CDI, but to overall acquisition of *C. difficile*. No studies to date have looked at the individual patient characteristics/risk factors for short-term acquisition (<14 days) of CDI among hospitalized patients who are initially PCR negative. As repeat testing is costly, knowing which patients would benefit from repeat testing could be used to guide laboratory policies (12), yet still allow for cases of short-term CDI to be diagnosed. This study sought to determine if there are identifiable antibiotic and patient comorbidities associated with positive repeat PCR tests after an initial negative test. If these could be identified, they could be used to develop algorithms that would improve the efficiency of a short-term repeat testing protocol for detecting *C. difficile.* Practically speaking, this would deliver cost benefits for healthcare quality by decreasing the cost of testing.

The objective of this study was to determine the rate of short-term acquisition (within 14 days) of *C. difficile* infection after an initial negative PCR, and to determine patient comorbidities and antibiotic use associated with incidence in these hospitalized patients.

BACKGROUND

Clostridium difficile was not identified as the causative bacterial agent for antibiotic-related diarrhea until the late 1970s (4, 13, 14) and is now recognized as the leading cause of hospital-acquired diarrhea (4, 15). Prior to 1978 when this organism was cultured and the toxin effects were demonstrated in the intestines of rodent models (14), *Staphylococcus aureus* was thought to be the cause of pseudomembranous colitis (13). Humans are able to co-exist with this organism as long as there is no disruption in the microbiota of the intestine (16). Hospitalizations and deaths from *C. difficile* have doubled in the past 10 years (17, 18), and the healthcare costs have skyrocketed as a result of these infections. The length of stay for an individual with effectively doubles (11). In the last decade (since 2003), there have been increasing rates of CDI in North American and Europe, with a larger proportion of severe or recurrent cases (19).

The gold standards for the detection of the *C. difficile* toxin are the toxigenic stool culture or cytotoxicity assay; however, these tests are impractical for clinical laboratories. Most clinical laboratories eventually implemented the EIA to detect toxin in the stool samples, and traded efficiency for poorer sensitivity (20). In 1995, Manabe et al. reported the need to test successive stool specimens in order to increase the diagnostic yield of the EIA (21). They found that 72% of patients with *C. difficile* were positive by EIA on the first run and that 10% of positive patients were missed by EIA compared to tissue culture cytotoxicity assay. In 1997, practice guidelines were published that stated that when *C. difficile* is clinically suspected, a single stool specimen should be sent for testing; however, if the result is negative, one to two additional stools should be sent for retesting (22). Performing EIA on two or three specimens, rather than one, increased the diagnostic

yield by 5 to 10 percent, but also increased the cost (13). This impression that all testing for *C. difficile* is insensitive has led clinicians to test multiple samples (repeat testing) in an attempt to improve diagnostic accuracy. The detection of this organism has changed rapidly in the last few years with the advent of molecular testing with PCR, which detects the gene that produces the toxin. Current guidelines suggest three tests for the rapid diagnosis of CDI: enzyme immunoassay (EIA), glutamate dehydrogenase detection (GDH) algorithm, and PCR testing (17, 23, 24), with PCR being the most sensitive.

A history of antibiotic use within the previous 8 weeks is usually present for the majority of patients (25, 26), and it has been shown that there is a 7 to 10-fold increased risk for *C. difficile* infection (CDI) during antibiotic therapy and in the first month after antibiotic completion (27). Specific antibiotics that lead to CDI have been implicated recently in two meta-analyses, which reported the greatest risk of CDI to be associated with clindamycin use, followed by fluoroquinolones or cephalosporins (28, 29). At moderate risk for CDI were the sulfonamides, macrolides and penicillins. Tetracyclines were shown to not increase the risk for CDI (28, 29). Since the risk of CDI is tied to antibiotic use in 90% of patients, and given these recent data that the antibiotic class itself may be implicated, this study also determined to look at the individual classes of antibiotics that were administered 8 weeks prior to the first negative test and 14 days after this test for risk of short-term acquisition of CDI.

Underlying patient comorbidities for acquisition of *C. difficile* include advanced age (30), immunosuppression (chemotherapy (31), neutropenia (32), HIV(33)), recent gastrointestinal (GI) surgery (34), tube feeding (35) and potentially the use of proton pump inhibitors (PPI) (36). Duration of hospitalization (1, 17) is also a significant risk

factor for acquisition of CDI. In one study, the presence of *C. difficile* spores in the hospital environment was found on 49% of surfaces in rooms occupied by patients with CDI and on 29% of surfaces in rooms of asymptomatic carriers (37). Indeed, a recent study reported that the adjusted hazard of CDI among patients who stayed in a hospital room previously occupied by a CDI patient was 2.35 times that of patients staying in a room where the previous occupant was not a CDI patient (38). It follows that the longer an individual is hospitalized in this healthcare environment, the more risk they are at for contracting *C. difficile* spores (37).

The median onset of symptomatic infection after colonization with toxigenic *C. difficile* spores is typically 2-3 days (39). Given the high sensitivity of PCR testing and the low incidence of short-term acquisition of CDI, most institutions have recommend against repeat testing within 7 days of a negative test (6). While previous studies show that short-term acquisition (within 14 days) of CDI in patients with repeat testing who had an initial negative PCR is rare (1-4%) (5, 6, 40, 41), whether specific patient characteristics place subgroups of patients at increased short-term risk of CDI remains understudied. This subset of patients with short-term acquisition who have a positive PCR on repeat testing after an initial negative are exclusively hospitalized patients (5, 6), and for this reason formed the cohort to be studied. Since repeat testing is costly, determining which patients would likely benefit from repeat testing could be used to guide laboratory policies (12). The clinical syndromes of this disease vary from diarrhea to severe sepsis leading to an entity called toxic megacolon, which can lead to death (13). Patients at the highest risk of morbidity and mortality with this organism include the immunocompromised, such as organ transplant patients (42) and the elderly (8).

Although infection with *C. difficile* accounts for only 10 to 20 percent of the cases of antibiotic-associated diarrhea, it accounts for the majority of cases of colitis associated with antibiotic therapy (13). The goal of this study was to determine if there are identifiable antibiotic use and patient comorbidities that would improve the efficiency of a short-term repeat testing protocol for detecting *C. difficile* in the clinical laboratory.

METHODS

Research Goal

Since the short-term risk of acquisition of CDI after a negative PCR test is very low, and repeat testing is inefficient and costly, the research goal is to determine if there are patient characteristics (antibiotic use and comorbidities) that are associated with a higher risk for the development of CDI within 14 days after a negative test. Specifically, this study will 1) estimate the short-term rate of acquisition of *C. difficile* among hospitalized patients who have an initial negative PCR test and received subsequent tests, and 2) estimate the association between selected factors found in hospitalized patients (antibiotic use and comorbidities) and short-term risk of *C. difficile* infection.

Research Question

Are there are identifiable characteristics, specifically of antibiotic use or patient comorbidities that would improve the efficiency of a short-term repeat testing protocol for detection of *C. difficile* in hospital patients?

Specific Aims

1) Estimate the short-term rate of acquisition of *C. difficile* among hospitalized patients who have an initial negative test and received subsequent tests
2) Estimate the association between selected comorbidity and antibiotic use factors found in hospitalized patient and the short-term risk of *C. difficile* infection

Study Design and Population

A matched case-control study was conducted at a university-affiliated healthcare system in a large metropolitan area in the southeast United States. The clinical microbiology section of Emory Medical Laboratories (EML) performs PCR testing for *C. difficile* for the following healthcare facilities in metropolitan Atlanta: The Emory Clinic, Emory University Hospital, Emory University Midtown, Emory University Orthopaedics and Spine Hospital, Emory-Adventist Hospital at Smyrna, Wesley Woods Center, and Emory Johns Creek Hospital. The transplant program at Emory University Hospital performs approximately 350 solid organ transplants and 150 hematopoietic stem cell transplants annually, and functions as a regional referral center for the treatment of patients with malignancies, particularly leukemia and lymphoma.

Consecutive adult patients who had a stool sample sent to the clinical microbiology section for PCR testing for *C. difficile* from November 2010 to September 2012 were eligible for the study. Adults were defined as >18 years of age. Study patients were defined as short-term acquisition of CDI cases if they initially tested *C. difficile* PCR negative followed by a positive PCR test within 14 days of the negative test. The duration of 14 days was chosen due to the precedent in the literature as described above (5, 6, 40, 41). The controls were individuals who had repeat testing within 14 days and both the initial and repeat tests were negative. To determine the association of selected patient characteristics with short-term risk of CDI, cases were matched to three randomly selected controls by 1) days of hospitalization to first *C. difficile* PCR test (±1 day) and 2) age (range ± 10 years). These variables were chosen for matching given that they were potential confounders for risk of CDI. Controls (90 total) were selected randomly from a

pool of the hospitalized patients whose initial PCR tests for *C. difficile* were negative during the same study period as cases, and had repeat negative PCR tests (and never tested positive) within 14 days. Therefore, in the underlying cohort of 750 patients with repeat PCR testing for *C. difficile*, all of the eligible cases were selected (30/30, 100.0%), and for controls, 90 were selected randomly and matched as above, and represent 12.5% (90/720) of the underlying cohort who did not acquire *C. difficile* in 14 days.

Data Collection and Laboratory Procedures

All stool samples sent to the clinical microbiology laboratory for *C. difficile* testing were processed, according to the manufacturer's instructions, using the Xpert® *C. difficile* test (Cepheid, Sunnyvale, CA) which detects the presence of toxin B gene (43). Emory University Institutional Review Board approval was obtained for retrospective chart review. Trained study staff performed retrospective medical chart reviews to obtain patient information on baseline demographics, clinical characteristics and co-morbidities. Clinical variables collected included antibiotic use or a gastrointestial procedure 8 weeks prior to the first negative test, proton-pump inhibitor (PPI) therapy use within 7 days prior to the first negative test, chronic steroid use (≥ 10 mg daily prednisone for ≥ 3 months), intensive care unit (ICU) admission within 7 days prior to testing and/or 48 hours after testing, and concurrent co-infections (either gastrointestinal or systemic infections other than *C. difficile*). Variables that were collected specifically for the period after the first negative test include ICU admission, as listed above, antibiotics during the 14 day time period after the first negative test, and treatment for CDI (Figure 1). All data were entered into a REDCap electronic database (44). REDCap is a secure, web-based

application that allows validated data entry with quality control and export procedures into statistical software.

Statistical Analyses

Data were analyzed using SAS® version 9.3 (Cary, NC) and OpenEpi 2.3.1 (Open Source Epidemiologic Statistics for Public Health, http://www.openepi.com). The rate of short-term acquisition of CDI after a first negative test was calculated using the number of cases divided by the person-days of the entire hospitalization of patients with a first negative PCR test (those whom were at risk, i.e, had a negative test). This calculation assumed that all other individuals in the hospital who were not tested via PCR over the study period were not at risk for CDI. The interval for those at risk included the sum of the person-days of the cases until the date of the first positive PCR in addition to the total hospital days of the individuals who had repeat testing (the denominator of the above rate). A χ^2 test was used to assess the association between antibiotic use and comorbidities and short-term CDI. A two-sided *p*-value less than 0.05 was considered statistically significant. In multivariable analyses, conditional logistic regression was used to estimate the association between the patient comorbidities (i.e., immunosuppression, gastrointestinal procedure, ICU stay) or antibiotic class and short-term acquisition of *C. difficile* controlling for the matched patient characteristics of age and days of hospitalization to first PCR test.

RESULTS

During the study period, a total of 12,021 *C. difficile* PCR tests were performed and of those, 9,312 PCR tests were excluded because those patients only received a single test (Figure 1). Of the 2,709 tests that remained, 430 PCR tests were further excluded because the repeated testing was performed after a first positive test. Of the 2,279 that had a repeat PCR test after a first negative PCR, 750 were within 14 days. Among hospitalized patients who initially tested *C. difficile* negative, 30 of 750 patients (4.2%, 95% confidence interval [CI] 2.88 - 5.82%) had an initial negative PCR followed by a positive PCR within 14 days (cases). For fifteen (50%) of the 30 cases of acquired CDI, the first positive PCR test occurred within 7 days of the initial negative PCR test, which is within the window that repeat testing is typically rejected from the laboratory (6). The rate of short-term acquisition of CDI in the study population was 142 per 100,000 person years (CI 97-200 per 100,000 person years). All of the patients in the study who became positive within the 14 days had true clinical disease by chart review by established definitions (17).

The success of the matching for age and days to first PCR test for the 90 controls that were selected out of the 720 non-cases for the case-control study is shown in Table 1. The mean age in the cases was 58.8 years ± 13.5, and for the controls was 58.6 years ± 13.5. The mean days to first test for cases was 7.8 days ± 5.8 and for the controls was 7.8 days ± 5.7.

In the 120 patients studied (30 cases and 90 controls), 52.5% (63/120) were male, 47.5% (57/120) were female, mean age was 59 (range 25-88) years (Table 1), and the mean hospital stay was 29.5 (range 4.0 – 143.0) days. Twenty-nine (24.1%) had diabetes

mellitus (DM), 17 (14.1%) had end-stage renal disease (ESRD), 39 (32.5%) had a hematologic malignancy (leukemia or lymphoma), 14 (11.6%) had a solid tumor, 11 (9.1%) were recipients of solid organ transplants, and 14 (11.6%) died during their hospital stay. Among the 120 study patients, PPI therapy (85.0%), recent (GI) procedure (16.7%), antibiotic use (91.6%), and admission to the ICU (45%) were common. No statistically significant differences were detected between the short-term CDI cases and matched controls in terms of patient demographic or clinical characteristics (Table 1), however, a suggestive association was found for recent GI surgery (26.6% vs. 13.3%; p=0.09). Cases were more likely to be male (63.3% vs. 48.8%), have had a recent GI procedure (26.6% vs. 13.3%) and less likely to have leukemia (23.3% vs. 35.5%), although these differences were not statistically significant.

Antibiotic use in the 8 weeks prior to the first negative PCR test differed among the cases and controls (Table 1). Compared to controls, cases more likely to be treated with vancomycin (66.7% versus 38.9%; p=0.009), and less likely to be treated with antiviral medication (20.0% vs. 44.4%; p=0.02). No patients in the cases or controls were on tetracycline, clindamycin, aztreonam, or daptomycin in the 8 weeks prior to the first negative PCR test. Antibiotic use in the 14 days after the first negative PCR test also differed between the cases and controls. Cases were more likely than the controls to be on oral vancomycin (16.6% versus 3.3%; p=0.01) or metronidazole (33.3% versus 16.7%; p=0.05), which are the treatments for CDI (17). The category of other included 5 patients who were on the same antibiotics and were kept on these after the first negative PCR test.

After adjusting for age and days of hospitalization prior to first PCR test, no patient characteristics were associated with cases or controls (Table 3). In adjusted analysis, cases were more likely to have end-stage renal disease (ESRD) (OR 1.71, CI 0.60-4.19), and be patients with recent GI procedure (OR 2.41, CI 0.84-6.88), but the detected differences were not statistically significant. No patients in the cases or controls were on tetracyclines, clindamycin, aztreonam, or daptomycin in the 8 weeks prior to the first negative PCR test. Antibiotic use in the 14 days after the 1st negative PCR test was different between the cases and controls in that the cases (who had been diagnosed with a positive PCR test) were more likely than the controls to be on oral vancomycin (5 (16.6%) versus 3 (3.3%); p=0.01) or metronidazole (10 (33.3%) versus 15 (16.7%); p=0.05), which are the treatments for CDI (17). The category of other included 5 patients who were on the same antibiotics and were kept on these after the 1st negative PCR test. One patient was receiving dapsone for *Pneumocystis jiroveci* prophylaxis, and another was being treated for disseminated *Mycobacterium avium* complex with azithromycin and ethambutol. The other patients were on meropenem, nitrofurantoin, and tigecycline.

In multivariable analyses, using conditional logistic regression, the patient comorbidities that were abstracted were not significantly associated with CDI (Table 3). After controlling for age and days of hospitalization prior to first PCR test, short-term acquisition of CDI was more common among PPI users (OR 1.18, CI 0.37-3.73), end-stage renal disease (ESRD) patients (OR 1.71, CI 0.60-4.19), and patients with recent GI procedure (OR 2.41, CI 0.84-6.88).

When comparing antibiotic use before the first performed PCR test (Table 4) the use of previous intravenous vancomycin was higher among cases than controls (OR 3.38,

CI 1.34-8.49), while the use of acyclovir for prophylaxis was more common among the controls (OR 0.30, CI 0.11-0.83)(Table 5). When intravenous vancomycin therapy was combined with a beta-lactam antibiotic, indicating that the patients were receiving both either together or separately, in the 8 weeks prior to the first PCR test, there remained a higher proportion in cases with incident CDI as compared to controls (OR 2.72; CI 1.10-6.72). Among cases, when intravenous vancomycin use in the 8 weeks prior to first PCR test is combined with a beta-lactam and a quinolone (typical empirical combination, and receiving them together or separately within the same time period), there is also a higher proportion of those with short-term acquisition as compared to controls (OR 2.60; CI 1.05-6.46). There was not a statistically significant different proportion of use between cases and controls when beta-lactam or quinolone antibiotics are considered individually in the 8 weeks prior to the first PCR test.

The number of individuals in the cases and controls who were on antibiotics, whether 8 weeks prior to the 1st negative test or within 14 days after the date of this test were identical (data not shown), which suggested that these patients remained on the same antibiotic regimen at the time of the first negative PCR test and then subsequently during the time of short-term acquisition of *C. difficile.*

DISCUSSION

In this case-control study, the short-term incidence rate of acquisition of CDI among hospitalized patients was 142 per 100,000 person years (CI 97-200 per 100,000 person years). Intravenous vancomycin (within 8 weeks prior to first PCR negative test) was more common among cases than controls (OR 3.38, 95% CI 1.34-8.49), even after adjusting for age and length of hospitalization. In addition, cases were less likely to have a history of acyclovir prophylaxis (OR 0.30. 95% CI 0.11, 0.83). However, clinical patient comorbidities that have been previously shown to be associated with CDI did not differ between cases and controls in this study (Table 3).

The rate of short-term acquisition in this study is comparable to the crude incidence rates in other studies (45), and the percentage of PCR tests (4.2%) that were initially negative and subsequently positive within 14 days also is similar to other studies (2.1-3.4%)(5, 6). The finding of prior intravenous vancomycin as a risk factor for incident CDI has been described (46), and one study attributed a relative risk of 18.2 for incident CDI (CI 14.2-23.3) if vancomycin was administered for >7days (47). The recent meta-analyses on antibiotic classes and their risk of incident CDI were on community-associated CDI (28, 29); the hospitalized patient cohort in this study is different, with almost no patients on antimicrobials (such as clindamycin) that are typically given as an outpatient. Patients in this study were more likely to be on intravenous antibiotics, and were being exposed to the hospital reservoir of *C. difficile* than the general population. Given this finding, the assumption was that the increased odds associated with vancomycin might have been a surrogate for poly-antimicrobial use. However, the two groups were similar in the percentage of patients on 1,2,3 or 4 antibiotics 8 weeks prior to

the first negative test or 14 days afterward (data not shown). There also was a higher proportion of control patients after the first negative test who were on >4 antibiotics. Studies that have looked at antibiotic use in hospitalized patients have found prior administration of clindamycin and beta-lactams to be implicated in acquisition of *C. difficile* (48), while others have focused on the association of CDI with a summation of prior therapy with certain antibiotic classes. The data in this study generates a hypothesis suggests that there may be a subgroup of intravenous antibiotics that may put hospitalized or hospital-experienced individuals at higher risk.

The finding that intravenous vancomycin was associated with CDI could be partially explained by the fact that more cases were likely to have been in the hospital prior to their current hospitalization in order to have received an intravenous vancomycin. These individuals also had substantial underlying comorbidities in addition to being healthcare-experienced. It has been demonstrated that antibiotic perturbation is necessary for dysbiosis that allows *C. difficile* to causes disease (16, 49). Therefore, it follows that chronic comorbidities that may require frequent antibiotic use, hospital exposure to the spores, or immune compromise puts individuals at risk for acquisition of CDI. It is likely that the strong association with vancomycin found in this study may indeed be a surrogate for individuals who are more chronically ill (50). Vancomycin can also be used for surgical prophylaxis (possible confounding by indication), but in this study it was used in patients with culture-directed infections or as empirical use in febrile syndromes in complicated patients. In this population studied, almost half were in the ICU during their hospitalization in which they received repeated PCR tests for *C. difficile*. While intravenous vancomycin itself is not used as a therapy for *C. difficile* because of the low

concentrations found in stool, it has been shown to be associated with altered microbiota, specifically vancomycin-resistant enterococci (51). While vancomycin may not be in the causal pathway of short-term acquisition of *C. difficile,* it is clearly important an important marker of risk of short-term acquisition.

Limitations

A limitation of this study is that there may have been residual confounding resulting from some of the variables that were not collected during chart review. The study did not look at the summation or severity of comorbidities, their overall healthcare experience previously (only current LOS), or long term duration of antibiotic use. The duration of antibiotics that each individual was on were also not extracted, which would have been used to determine if duration of antibiotic treatments (besides the type of antibiotic) was a factor in short-term risk of CDI. Given that antibiotics were given to >90% of patients in this study, there may have also been confounding by indication, given the variety of syndromes for which the patients were hospitalized. We also found that acyclovir use among cases was at a lower proportion than among controls, which appeared protective. However, the underlying distribution of previous acyclovir among cases and controls was also not proportional (20.0% vs 44.4%; p=0.02). The underlying cohort of 750 individuals from which we drew the cases and controls, in order to be tested twice within 14 days, included individuals who were hospitalized for lengthy periods of time, such as hematopoietic stem cell transplant patients. We randomly over-sampled a group that used acyclovir for prophylaxis.

There was a potential in this study for the misclassification of exposure. We had two individuals who performed chart review, but we did not cross check the abstracted data. In theory, there may have been misclassification by outcome (i.e. colonization by *C. difficile* (17)), but PCR has extremely high sensitivity and specificity, especially in the setting of true clinical disease, which was documented in these case patients.

The probability of a type II error was potentially higher due to a low sample size. The sample size, while small, does exceed most studies that have looked at patients who have had a 2nd PCR test that was positive after a 1st negative within 14 days (Table 7). Also, the policy of this laboratory is to automatically reject stools that are submitted within 7 days, unless the practitioner calls back to request. This may introduce a selection bias but more than likely enriches the population study for those who are likely to be suspected to be ill enough to warrant repeat testing.

Implications

The typical practice in the clinical microbiology laboratory is to reject stool samples that are sent for *C. difficile* PCR within 7 days. This interval is arbitrary, especially given that the incubation period for CDI is not precisely known. Samore et al showed that the period of shedding in stool after initial infection of *C. difficile* was 3-7 days (39). This study attempted to identify risk factors in those individuals who acquired CDI within 14 days of a negative test. Fourteen days was chosen initially given the precedent in the literature (5, 6, 41), and in this study, 30 individuals had acquisition of CDI within 14 days of their first negative test, and 15 (50%) of those were within 7 days. From 2010 to 2012, this institution had a CDI rate of 0.0014 persons/day in hospitalized

patients within 14 days of a negative test among patients who had subsequent tests after an initial negative test. This rate is similar to what has been described in the literature (5). This case-control study did not yield any patient comorbidities that were significant except for a trend towards risk of short-term acquisition in patients with recent GI procedure, which has been described as a risk factor for *C. difficile* in general (34, 35, 52, 53). The hypothesis for CDI in patients with a recent GI procedure is that these patients 1) may have been infected by exposure to environments highly colonized with *C.difficile*, 2) have had alteration of the fecal flora due to manipulation or 3) may be more likely to be ill or debilitated. The typical risk factors (Table 1) for *C. difficile* did not differ between the cases and controls.

Given the recent information in the 2 meta-analyses that demonstrated that there are different risks for CDI given antibiotic class (28, 29), this study sought to determine if a certain class of antibiotic use showed increased risk of specifically short-term acquisition of CDI. The patient population in this study was quite different than the meta-analyses population, since the meta-analysis included all-comers, whereas this study has only hospitalized patients. The patients in this study also tended to be on intravenous antibiotics, and were actively exposed to the hospital reservoir of *C. difficile*. Therefore, these were patients who did not receive the typical antibiotic courses that would have occurred in the outpatient setting, such as clindamycin.

Intravenous vancomycin use has not been examined in the literature as a specific risk factor for *C. difficile*, and may be a coincidental factor more than a causal one. Patients who have received intravenous vancomycin in the last 8 weeks are likely hospital-experienced and have significant chronic illnesses, both of which are risk factors

for CDI (9). This association did not hold true when intravenous vancomycin was given after the first negative test and prior to the repeated testing. This supports that use of intravenous vancomycin in the 8 weeks prior to the first test is likely a surrogate for a patient who is already high-risk for contracting *C. difficile*. However, the two groups were similar in the percentage of patients on 1,2,3 or 4 antibiotics 8 weeks prior to the first negative test or 14 days afterward (Table 6), but vancomycin was still associated despite the similar number of antibiotics used. There also seemed to be a higher proportion of control patients after the first negative test who were on >4 antibiotics (Table 6). Previous intravenous vancomycin use remains an important association with short-term CDI in hospitalized patents despite the majority (90%) of cases and controls being on antibiotics.

Although it is clear that the great majority of patients do not need repeated *C. difficile* testing within 14 days, there are still individuals who test positive within this timeframe, and this is an important diagnosis to make (4). Current guidelines do not support repeated testing for either EIA assays or PCR testing (17). The goal was to determine by case-control methodology if there were individual patient comorbidities that could be generalized for short-term acquisition of CDI. Since this tertiary referral center has a high number of patients who are at risk for incident CDI, a subset of patients who are at even a higher risk could not be determined.

In conclusion, intravenous vancomycin use within the 8 weeks prior to the first test for CDI was predictive of short-term acquisition of *C. difficile* in hospitalized patients. The practical implications for this in terms of repeated testing may include

eliciting this antibiotic history when clinicians request testing earlier than 7 days in a hospitalized patient.

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Figure 1: Timing of patient characteristics in retrospective chart review

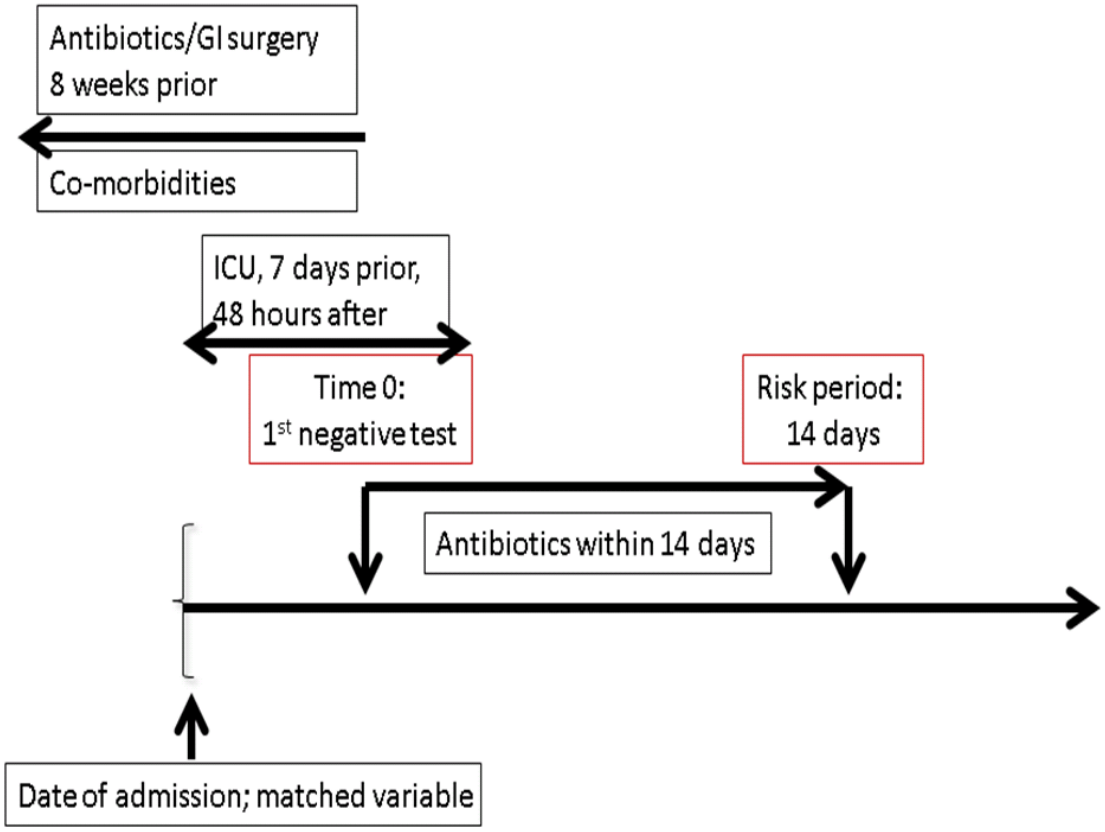

Figure 2: Patient selection flowsheet

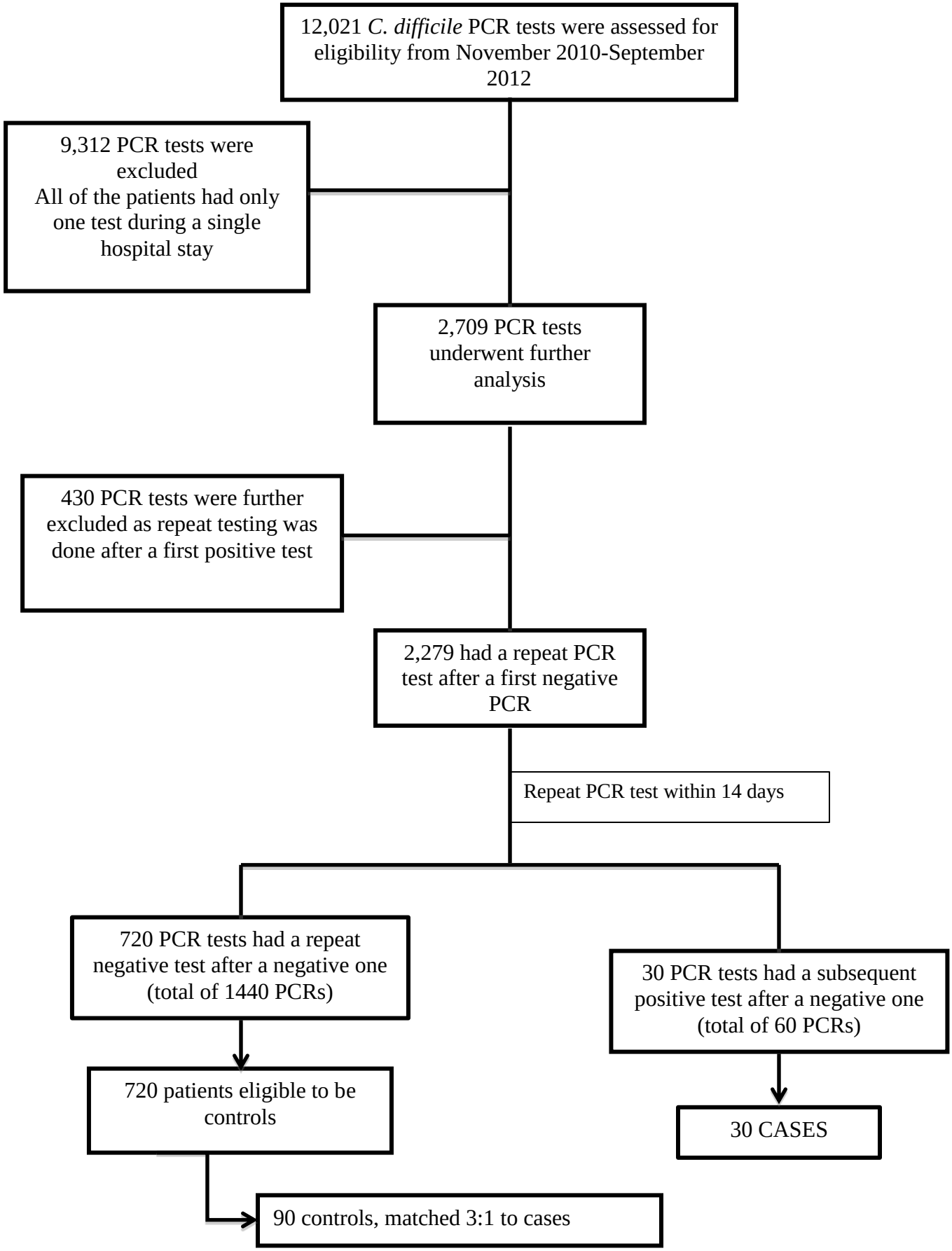

Table 1. Factors associated with short-term acquisition of nosocomial *C. difficile* infection among hospitalized patients, 2010-2012				
Characteristics	**Cases N (%) N= 30**	**Controls N (%) N= 90***	**Total N (%) N= 120**	**P-value[1]**
Days to 1st test[2](matched)	7.8 ± 5.8	7.8 ± 5.7	--	--
Age (in years) (matched)	58.8 ± 13.5	58.6 ± 13.5		
Gender				
Female	11 (36.6%)	46 (51.1%)	57 (47.5%)	0.17
Male	19 (63.3%)	44 (48.8%)	63 (52.5%)	
Hospital stay				
Mean (STD)	33.06 (26.8)	28.3 (17.8)		0.28
Median (IQR)	27 (18)	22.5 (18)		
Death (all causes)	5 (16.6%)	9 (10%)	14 (11.6%)	0.33
Antibiotic (after 1st PCR)				
Yes	27 (90%)	83 (92.2%)	110 (91.6%)	0.70
No	3 (10%)	7 (7.7%)	10 (8.3%)	
ICU stay[3]				
Yes	11 (36.6%)	43 (47.7%)	54 (45%)	0.29
No	19 (63.3%)	47 52.2%)	66 (55%)	
Diabetes Mellitus				
Yes	7 (23.3%)	22 (24.4%)	29 (24.1%)	0.90
No	23 (76.6%)	68 (75.5%)	91 (75.8%)	
Co-infection[4]				
Yes	18 (60%)	47 (52.2%)	65 (54.1%)	0.46
No	12 (40%)	43 (47.7%)	55 (45.8%)	
PPI use				
Yes	26 (86.6%)	76 (84.4%)	102 (85%)	0.77
No	4 (13.3%)	14 (15.5%)	18 (15%)	
Recent GI procedure[5]				
Yes	8 (26.6%)	12 (13.3%)	20 (16.6%)	0.09
No	22 (73.3%)	78 (86.6%)	100 (83.3%)	
ESRD				
Yes	6 (20%)	11 (12.2%)	17 (14.1%)	0.29
No	24 (80%)	79 (87.7%)	103 (85.8%)	
Leukemia				
Yes	7 (23.3%)	32 (35.5%)	39 (32.5%)	0.22
No	23 (76.6%)	58 (64.4%)	81 (67.5%)	
Solid tumor				
Yes	5 (16.6%)	9 (10%)	14 (11.6%)	0.33
No	25 (83.3%)	81 (90%)	106 (88.3%)	
Chemotherapy[6]				
Yes	10 (33.3%)	31 (34.4%)	41 (34.1%)	0.91
No	20 (66.6%)	59 (65.5%)	79 (65.8%)	

Stem cell transplant				
Yes	1 (3.3%)	1 (1.1%)	2 (1.6%)	0.41
No	29 (96.6%)	89 (98.8%)	118 (98.3%)	
Solid organ transplant				
Yes	2 (6.6%)	9 (10%)	11 (9.1%)	0.58
No	28 (93.3%)	81 (90%)	109 (90.8%)	

† Bold signifies statistical significance

* Controls were individual matched to cases by age and days before first PCR test. Two of the cases needed to be paired with 2 controls outside the age range (±15 years).

[1]Two-sided p-values <0.05 were considered significant

[2]Days of hospitalization prior to first *C. difficile* PCR test

[3]Intensive care unit stay during the same hospital admission

[4]Infection of any type and any source at the time of PCR testing

[5]Gastrointestinal procedure of any type within 8 weeks prior to 1st PCR test

[6]Received chemotherapy for malignancy within the last 8 weeks

Table 2. Specific antibiotic use 8 weeks prior and 14 days after 1st negative PCR test				
Characteristics	**Cases N (%) N= 30**	**Controls N (%) N= 90***	**Total N (%) N= 120**	**P-value[1]**
8 weeks prior to PCR test				
Beta-lactam	22 (73.3%)	50 (55.6%)	72 (60.0%)	0.09
Macrolide	2 (6.7%)	5 (4.2%)	7 (5.8%)	0.82
Quinolone	14 (46.7%)	40 (44.4%)	54 (45.0%)	0.83
Aminoglycoside	1 (3.3%)	6 (6.7%)	7 (5.8%)	0.50
TMP-SMX	5 (16.7%)	11 (12.2%)	16 (13.3%)	0.54
Metronidazole (PO/IV)	2 (6.7%)	9 (10.0%)	11 (9.2%)	0.58
Vancomycin IV	20 (66.7%)	35 (38.9%)	55 (45.8%)	**0.009**
Vancomycin PO	2 (6.7%)	1 (1.1%)	3 (2.5%)	0.09
Antifungal	10 (33.3%)	41 (45.6%)	51 (42.5%)	0.25
Antiviral	6 (20.0%)	40 (44.4%)	46 (38.3%)	**0.02**
Linezolid	1 (3.3%)	3 (3.3%)	4 (3.3%)	1.00
Other[2]	2 (6.7%)	3 (3.3%)	5 (4.2%)	0.43
14 days after PCR test				
Beta-lactam	19 (63.3%)	62 (68.9%)	81 (67.5%)	0.58
Macrolide	1 (3.3%)	4 (4.4%)	4 (4.2%)	0.79
Quinolone	12 (40.0%)	33 (36.7%)	45 (37.5%)	0.75
Aminoglycoside	3 (10.0%)	6 (6.7%)	9 (7.5%)	0.55
TMP-SMX	2 (6.7%)	5 (5.6%)	7 (5.8%)	0.83
Metronidazole (PO/IV)	10 (33.3%)	15 (16.7%)	25 (20.8%)	**0.05**
Vancomycin IV	9 (30.0%)	43 (47.8%)	52 (43.4%)	0.09
Vancomycin PO	5 (16.6%)	3 (3.3%)	8 (6.7%)	**0.01**
Antifungal	11 (36.7%)	43 (47.8%)	54 (45.0%)	0.29
Antiviral	8 (26.7%)	37 (41.1%)	45 (37.5%)	0.16
Linezolid	0 (0.0%)	5 (5.6%)	5 (4.2%)	0.19
Tetracycline	0 (0.0%)	1 (1.1%)	1 (0.8%)	0.56
Clindamycin	0 (0.0%)	1 (1.1%)	1 (0.8%)	0.56
Aztreonam	0 (0.0%)	4 (4.4%)	4 (3.3%)	0.24
Daptomycin	1 (3.3%)	2 (2.2%)	3 (2.5%)	0.74
Other[2]	2 (6.7%)	3 (3.3%)	5 (4.2%)	0.43

*** Controls were individual matched to cases by age and days before first PCR test. Two of the cases needed to be paired with 2 controls outside the age range (±15 years).**

[1]Two-sided p-values <0.05 were considered significant

[2]Other antimicrobials included dapsone, meropenem, nitrofurantoin, ethambutol, tigecycline (one patient each)

Table 3. Conditional logistic regression of CDI for qualitative patient characteristics*		
Characteristic	Crude OR	Adjusted OR
Male gender	1.81 (0.77-4.22)	1.87 (0.76-4.18)
Death (all causes)	1.80 (0.55-5.87)	1.85 (0.54-6.28)
Antibiotic use after 1st PCR	0.76 (0.18-3.14)	0.75 (0.17-3.25)
ICU stay[1]	0.63 (0.27-1.48)	0.59 (0.24-1.45)
Diabetes mellitus	1.06 (0.40-2.81)	0.94 (0.35-2.47)
Co-infection[2]	1.37 (0.59-3.18)	1.49 (0.58-3.82)
Proton-pump inhibitor	1.20 (0.36-3.96)	1.18 (0.37-3.73)
Recent GI procedure[3]	2.36 (0.86-6.50)	2.41 (0.84-6.88)
End-stage renal disease	1.80 (0.60-5.36)	1.72 (0.60-4.19)
Leukemia	0.55 (0.21-1.43)	0.49 (0.17-1.38)
Solid tumor	1.80 (0.55-5.87)	1.85 (0.54-6.28)
Chemotherapy[4]	0.95 (0.40-2.28)	0.94 (0.37-2.40)
Stem cell transplant	3.07 (0.19-50.64)	3.00 (0.19-47.96)
Solid organ transplant	0.64 (0.13-3.16)	0.67 (0.14-3.09)

* Controls were individual matched to cases by age and days before first PCR test. Two of the cases needed to be paired with 2 controls outside the age range (±15 years).

[1]Intensive care unit stay during the same hospital admission

[2]Infection of any type and any source at the time of PCR testing

[3]Gastrointestinal procedure of any type within 8 weeks prior to 1st PCR test

[4]Received chemotherapy for malignancy within the last 8 weeks

Table 4. Conditional logistic regression of CDI by antibiotic class*		
Antibiotic, antifungal, and antiviral use 8 weeks prior to first PCR test		
Type of antibiotic	Crude OR†	Adjusted OR†
Beta-lactam	2.20 (0.88-5.46)	2.35 (0.91-6.07)
Macrolide	1.21 (0.22-6.61)	1.20 (0.23-6.19)
Quinolones	1.09 (0.47-2.50)	1.10 (0.47-2.59)
Aminoglycoside	0.48 (0.06-4.18)	0.50 (0.06-4.15)
TMP-SMX	1.43 (0.45-4.53)	1.43 (0.45-4.52)
Metronidazole	0.64 (0.13-3.16)	0.67 (0.14-3.09)
Vancomycin IV	**3.14 (1.31-7.49)**	**3.38 (1.34-8.49)**
Vancomycin PO	6.36 (0.56-72.77)	6.00 (0.54-66.17)
Antifungal	0.60 (0.25-1.41)	0.58 (0.24-1.41)
Antiviral	**0.31 (0.11-0.83)**	**0.30 (0.11-0.83)**
Linezolid	1.00 (0.10-9.99)	1.00 (0.08-11.93)
Other[1]	2.07 (0.33-13.03)	2.30 (0.31-17.24)
Antibiotic, antifungal, and antiviral use 14 days after first PCR test		
Type of antibiotic	Crude OR	Adjusted OR
Beta-lactam	0.78 (0.32-1.85)	0.81 (0.36-1.81)
Macrolide	0.74 (0.08-6.90)	0.75 (0.08-6.71)
Quinolone	1.15 (0.49-2.69)	1.18 (0.47-2.98)
Aminoglycoside	1.56 (0.36-6.65)	1.50 (0.38-6.00)
TMP-SMX	1.21 (0.22-6.61)	1.22 (0.22-6.94)
Metronidazole	2.50 (0.98-6.40)	2.39 (0.95-6.06)
Vancomycin IV	0.46 (0.19-1.13)	0.47 (0.19-1.16)
Vancomycin PO	**5.80 (1.30-25.96)**	**6.63 (1.27-34.74)**
Antifungal	0.63 (0.27-1.48)	0.64 (0.27-1.48)
Antiviral	0.52 (0.20-1.29)	0.51 (0.20-1.29)
Linezolid	0.25 (0.01-4.75)	-
Tetracycline	0.98 (0.39-24.6)	-
Clindamycin	0.98 (0.39-24.6)	-
Aztreonam	0.31 (0.02-6.02)	-
Daptomycin	1.52 (0.13-17.35)	1.50 (0.14-16.54)
Other[1]	2.07 (0.33-13.03)	2.00 (0.33-11.97)

***OR matched on age and days to first PCR test**

† Bold indicates statistical significance

[1]Other antimicrobials included dapsone, meropenem, nitrofurantoin, ethambutol, tigecycline (one patient each)

Table 5. Conditional logistic regression of CDI with combined antibiotic classes		
Antibiotic class administered 8 weeks prior to first test		
	Crude	Adjusted*
Beta-lactam	2.20 (0.88-5.46)	2.35 (0.91-6.07)
Quinolone	1.09 (0.47-2.50)	1.10 (0.47-2.59)
Vancomycin intravenous	**3.14 (1.31-7.49)**	**3.38 (1.34-8.49)**
Beta-lactam + vancomycin	**2.47 (1.06-5.76)**	**2.72 (1.10-6.72)**
Beta-lactam + vancomycin + quinolone	**2.36 (1.01-5.54)**	**2.60 (1.05-6.46)**
Antibiotic class administered 14 days after first test		
	Crude	Adjusted*
Beta-lactam	0.78 (0.32-1.85)	0.81 (0.36-1.81)
Quinolone	1.15 (0.49-2.68)	1.18 (0.47-2.98)
Vancomycin intravenous	0.46 (0.19-1.13)	0.47 (0.19-1.16)
Beta-lactam + vancomycin	0.48 (0.19-1.18)	0.49 (0.20-1.22)
Beta-lactam + vancomycin + quinolone	0.76 (0.33-1.75)	0.78 (0.35-1.73)
***OR matched on age and days to first test**		

Table 6. Percentage of patients with antibiotic use				
Category	**Cases N (%) N= 30**	**Controls N (%) N= 90**	**Total N (%) N= 120**	**P-value[1]**
Antibiotics 8 weeks prior to first test				
No antibiotics	3 (10%)	8 (8.9%)	11 (9.2%)	0.31
1,2 or 3 antibiotic classes	16 (53.3%)	61 (67.8%)	77 (64.2%)	
>4 antibiotic classes	11 (36.7%)	21 (23.3%)	32 (26.7%)	
Antibiotics 14 days after first test				
No antibiotics	3 (10.0%)	7 (7.8%)	10 (8.3%)	0.26
1,2 or 3 antibiotic classes	18 (60.0%)	45 (50.0%)	63 (52.5%)	
>4 antibiotic classes	9 (30.0%)	38 (42.2%)	47 (39.2%)	
*** Controls were individual matched to cases by age and days before first PCR test. Two of the cases needed to be paired with 2 controls outside the age range (±15 years).** **[1]Two-sided p-values <0.05 were considered significant**				

Table 7. Comparison of present study to previous studies with repeat testing for *C. difficile*. *Adapted from* Aichinger et al, 2008(5)

Reference	Date	Test	Total no. of patients/samples tested	No. of patients or samples with repeat testing	No. of tests converted from negative to positive
Manabe (21)	1995	EIA	268/692	162	9
Renshaw(10)	1996	Cell culture cytotoxicity	2,009/4,238	1,519	15
Morelli(40)	2004	EIA	130/147	63	1
Borek(54)	2005	Cell culture cytotoxicity	NR	1,101	2
Mohan(55)	2006	EIA	396/474	78	1
van den Berg(41)	2007	Enzyme-linked fluorescent assay	450/547	68	2
Aichinger(5)	2008	EIA, PCR	8,615/15,522	1,918	40
Luo(6)	2010	PCR	1,287/1,949	293	10
Present report	2013	PCR	12,021	750	30