Comparison of Clinical Features Between Inpatient and Outpatient Cases of *Clostridium difficile* Infection

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Abstract

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The Microbiology Laboratories at Harborview Medical Center and the University of Washington Medical Center evaluated the Biofire FilmArray Gastrointestinal Panel, a multiplex PCR assay to conventional stool culture. The FilmArray can detect both toxin A (*tcdA*) and toxin B genes (*tcdB*) in *Clostridium difficile*. *C. difficile* is not detected by conventional stool culture. Instead, both laboratories use the Cepheid GeneXpert *C. diffcile* assay to rapidly detect the toxin B gene (*tcdB*). These two different test methods and the testing requirements provided an opportunity to compare clinical features of patients whom CDI was detected by targeted testing to those whom CDI was an unexpected finding detected by the multiplex PCR assay. A retrospective observational cohort study was performed on one-hundred forty cases of diagnosed CDI. A comparison of risk factors, clinical presentation, and responses to CDI-specific therapy was done between inpatients and outpatient cases. Analysis of the results showed that inpatients and outpatients were considerably similar in all those categories. There is a significant proportion of the CDI burden, with potential of cases overlooked, in the outpatient setting.

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1. Introduction

Clostridium difficile is a spore-forming, obligate anaerobic, gram-positive bacillus that is implicated in a myriad of illnesses ranging from antibiotic-associated diarrhea, pseudomembranous colitis, megacolon, and other severe complications. Watery diarrhea that occurs three or more times in a 24 hour period is the hallmark clinical manifestation of *Clostridium difficile* infection (CDI). Pathogenesis is due to the production of toxins A and Toxin B, encoded by the *tcdA* and *tcdB* genes, after colonization with toxigenic strains of *C. difficile*. This colonization is most readily achieved when the healthy intestinal microbiota is disrupted, usually by exposure to antibiotics. These toxins are delivered into the cell and disrupt epithelial integrity, which leads to inflammatory changes in the bowel.[1]

C. difficile has several virulence factors including adhesins, a paracrytalline S-layer protein, fimbriae, flagella, and a capsule. Most important is the production of toxins A and B encoded by *tcdA* and *tcdB* that drive the pathology seen in CDI. Toxins A and B are two component proteins with an enzymatically-active A subunit and a B subunit that delivers A into the target cell. The toxin interacts with regulatory molecules and interrupts vital signaling pathways leading to cell rounding, shrinking, and death resulting in loss of the intestinal epithelial barrier. The toxins are glucosyltransferases that target intracellular GTP-binding proteins Rho, Rac, and Cdc42. These small GTPases are inactivated by glucosylation which disrupts the assembly and disassembly of the actin cytoskeleton. Inactivating Rho also leads to increased permeability of the intestinal epithelial cell.[30] The toxins also stimulate proinflammatory cytokines including IL-1β, TNF-α, and IL-8, inciting an inflammatory response. The genes *tcdA* and *tcdB* are found on the 19.6 kb pathogenicity locus (PaLoc). The PaLoc houses five genes including: *tcdC* (negative regulator), *tcdA* (toxin A), *tcdE* (encodes

protein that releases toxins), *tcdB* (toxin B), and *tcdR* (positive regulator). There is also a binary toxin, also known as Cdiff transferase (CDT), not located in the PaLoc. Binary toxin is a two subunit AB toxin that disrupts the cytoskeleton of the cell, resulting in cell rounding, loss of fluid, and cell death.[2]

The epidemiology of CDI has been changing over the last couple of decades. CDI has historically been considered a nosocomial infection acquired during an inpatient stay at a healthcare facility, but is now also commonplace in community settings. In 2011, Lessa et al. described the burden of CDI in the United States through population and laboratory-based surveillance across ten geographic locations. The estimated incidence of U.S. cases that year was 453,000, or 147.2/100,000 persons, comprising 293,300 healthcare-associated cases (95.3/100,000 persons), and 159,700 community-associated cases (51.9/100,000 persons).[3] CDI has continued to increase since 2011. The Centers for Disease Control and Prevention Emerging Infections Program (EIP) has continued to survey CDI, and in their 2015 annual report, CA-CDI showed a substantial increase with an estimated incidence of 65.8/100,000 persons.[30] This incidence level varies among different regions, with CDI annual incidence as high as 80 per 100,000 persons in some regions.[3,4] CA-CDI does not necessarily present in the same fashion as HA-CDI in terms of the risk factors for infection, making CDI a diagnostic challenge.

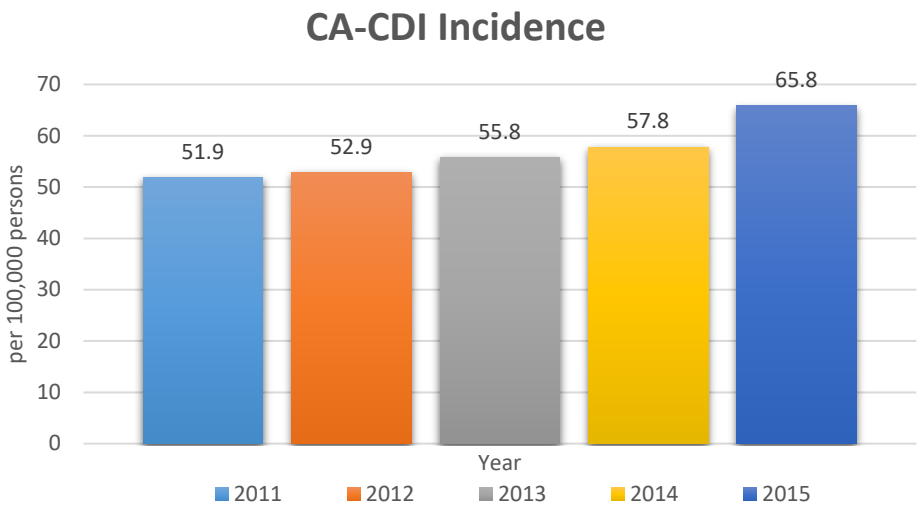


Figure 1. CDC EIP Surveillance CA-CDI Incidence from 2011 to 2015. [29]

2. Study Background

In January 2017, the Microbiology Laboratories at Harborview Medical Center (HMC) and the University of Washington Medical Center (UWMC) began an evaluation of the Biofire FilmArray Gastrointestinal (GI) Panel, a multiplex PCR assay, compared to conventional stool culture. When clinicians ordered testing for the detection of enteric pathogens, both the FilmArray and conventional stool culture were performed.

The use of a multiplex PCR assay allowed the comparison of targeted versus syndromic testing. Diarrhea can result from a variety of different pathogens that cause infectious gastroenteritis. A clinician may have sufficient information from the patient's history to choose a test that detects a specific pathogen. As an example, if a patient presents with diarrhea and has been using antibiotics to treat a recent infection, the suspicion is high for CDI, and clinicians

may elect to order a specific assay to detect *C. difficile* such as the GeneXpert *C. difficile* assay. However, if a patient presents with diarrhea and no specific epidemiologic risk factors, a clinician may prefer to obtain a multiplex PCR assay that simultaneously tests for several gastrointestinal agents. This is an example of syndromic testing, which can detect a variety of agents including unsuspected cases of CDI. Advantages to syndromic testing with multiplex PCR panels include reduced turn-around time, identification of co-infections, high negative predictive value that can avoid unnecessary infection control precautions, and high sensitivity. This enables a healthcare provider to make a prompt diagnosis and provide a patient with treatment appropriate for the specific microbial diagnosis. Laboratories can use PCR to replace slower, less sensitive, and more labor intensive tests such as culture with selective agars and enzyme immunoassays.[31, 32]

C. difficile is not detected by conventional stool culture, but the *tcdA* and *tcdB* are detected by the FilmArray GI panel. The targeted testing modality used at both hospitals for toxigenic *C. difficile* is the Cepheid GeneXpert *C. difficile* assay, which uses real-time PCR to rapidly detect the toxin B gene (*tcdB*). This test is typically ordered for inpatients who have been receiving antibiotic treatment. This differs from stool culture for inpatients because stool specimens from patients who have been hospitalized for more than three days are rejected. Conventional stool cultures tend to have a lower yield for detecting enteric pathogens in comparison to molecular assays, especially for fastidious bacteria such as *Campylobacter* spp. In addition, conventional culture methods fail to identify most diarrheagenic *E. coli* strains, which include enteropathogenic (EPEC), enterotoxigenic (ETEC), or enteroaggregative (EAEC) *E. coli* (STEC). *C. difficile* is not detectable by routine stool culture unless special selective media are incubated anaerobically.

The different test methods and the testing requirements provided a unique opportunity to compare patients in whom CDI was detected by targeted testing with those in whom CDI was an unexpected finding detected by the use of a multiplex panel. This retrospective observational cohort study was performed to compare inpatients and outpatients diagnosed with *C. difficile* infection. An additional aim was to determine the number of clinically significant cases of *C. difficile* infection detected by the Biofire FilmArray GI Panel as the only target detected.

3. Literature Review

The epidemiology of CDI has been evolving from what was once considered to be primarily a nosocomial infection to one encountered in community settings as well.[3,4] What makes this shift in epidemiology worrisome is that traditional risk factors seen in HA-CDI do not always apply to those seen in CA-CDI. Patients lacking traditional risk factors including healthy peripartum women, young adults and children, and patients who have not had antibiotic treatment or recent healthcare exposure have been diagnosed with CA-CDI, whereas HA-CDI is typically associated with older individuals (>65 in age) or those with recent or long-term antibiotic use and underlying co-morbidities.[5,6] CA-CDI patients tend to be younger and are more likely to be female than those with HA-CDI.[7,8] In order to better identify cases in the community setting, it would be useful to build a risk profile for CA-CDI.

In recent years, studies of HA-CDI and CA-CDI have profiled the similarities and differences amongst cases. HA-CDI is defined as a case with the onset of symptoms more than 48 hours after hospital admission. This is further sub-categorized by whether onset is in a healthcare facility or in a community setting. To be considered community onset, symptoms must present before 48 hours into an inpatient stay and less than four weeks from the last discharge from a healthcare facility. CA-CDI is defined as symptoms appearing in the

community or 48 hours or less into an admission to a healthcare facility and more than twelve weeks after the last discharge from a healthcare facility. Any cases occurring between four and twelve weeks after discharge are considered indeterminate.[3,6,8,9] This is demonstrated by Figure 2, which illustrates the categories of CDI along a timeline.

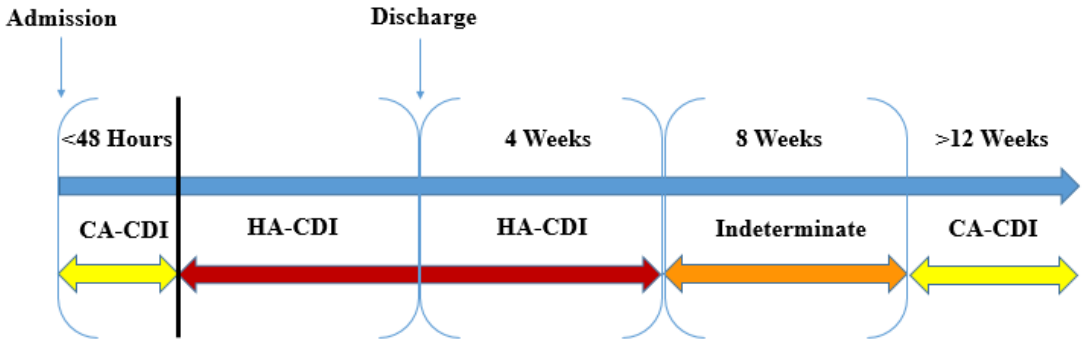


Figure 2. CDI Categories.
Timeline is subdivided into time of admission, four weeks, eight weeks, and more than twelve weeks after admission. CDI Categories are as follows: CA-CDI = Communty-Acquired CDI (Yellow Arrow), HA-CDI = Communty-Acquired *C.difficile* Infection (Red Arrow), Indeterminate (Orange).

The predominant risk factor associated with CDI is antibiotic exposure in both healthcare and community settings, particularly recent antibiotic exposure. Kutty et al. found extremely high risk of antibiotic exposure in a case-control study at a VA hospital (OR 7.8) and a local group of hospitals (OR 9.1).[10] The risk is highest at the time of therapy and decreases as time continues. This also holds true for recurrent cases of CDI, since normal intestinal flora may not fully repopulate after antibiotic exposure, allowing *C. difficile* to overgrow again.[11] Some antibiotic classes or drugs in particular may have a stronger association with CDI such as clindamycin, fluoroquinolones, and cephalosporins. These classes of antibiotics may pose more of a risk than macrolides, beta-lactam/beta-lactamase inhibitor combinations, and penicillins. [4-6,

[12] However, CA-CDI cases can lack traditional risk factors such as antibiotic use. Some studies have shown that a significant subset of CA-CDI patients, one third to one half, had not been prescribed an antibiotic within up to six months prior to presenting to a healthcare provider. [5,7,13]

Proton pump inhibitor (PPIs) use is associated with CDI. A meta-analysis of CDI risks found that PPIs were associated with an increased risk (Odds Ratio 1.69 - 1.74).[5] A case-control study in Japan compared CA-CDI patients to those who presented with diarrhea caused by a different etiology, and found no higher risk when patient were using PPIs[12], although this might signify that PPIs increase the risk of both CDI and other causes of enteritis. Some studies have found that PPIs are more of a risk factor when used long term, especially in patients with gastroesophageal reflux disease (GERD), with varying risk observed in HA-CDI and CA-CDI. [4,10,13] Reigidas et al. reported that PPIs are a major risk factor for recurrent CDI.[11] PPIs may limit stomach acidity so that *C. difficile* spores can survive and colonize the intestines, especially if the patient is also on antibiotics. A study that looked at the gut microbiome found that PPI users still had increased diversity of their microbiome compared to subjects on antibiotics, and the difference was more noticeable when greater numbers of antibiotics were prescribed.[14] The FDA has issued a warning that long-term use of PPIs can increase the risk of CDI.

Underlying comorbidities also play a role in CDI. For individuals that are immunocompromised or suffer from a chronic illness such as inflammatory bowel disease (IBD), GERD, chronic kidney disease (CKD), diabetes, malignancy or a solid organ transplant, the risk of acquiring CDI is higher.[4,6,15] Patients with HA-CDI tend to have more severe underlying illnesses, especially cardiovascular and malignant disease, whereas gastrointestinal illnesses are more common in those with CA-CDI.[11] Chemotherapy for cancer and corticosteroids or other immunosuppressive medications have been associated with CDI.[1-2]

First-line treatment for CDI consists of vancomycin or metronidazole, depending on CDI severity. Newer treatments include the antibiotic fidaxomicin or the fecal microbiota transplant (FTM) procedure. These can be considered when treatment failure occurs or when CDI recurs. Metronidazole is more often associated with treatment failure than vancomycin in complicated cases of CDI. Failure of first-line treatment in CA-CDI is more often seen in older patients and in those with severe CDI, underlying comorbidity and previous healthcare or antibiotic exposure.[1, 16]

Rapid diagnosis of CDI using molecular platforms is increasingly available. Broad reviews of the performance of different polymerase chain reaction (PCR) assays for the detection of toxigenic *C. difficile* show high sensitivity and specificity with 86 to 92% sensitivity and 94 to 97% specificity.[1] Conventional diagnosis by toxigenic culture or cell cytotoxicity assays, with or without the use of screening *C. difficile* glutamate dehydrogenase (GDH) tests, requires much longer turnaround times; cytotoxicity assays also suffer from low sensitivity. Enzyme immunoassays (EIA) are used for rapid diagnosis, but also exhibit poor sensitivity.[1] Many groups suggest using a multi-step approach or stand-alone PCR for the best sensitivity and specificity.[1] Although some argue that the high sensitivity of today's molecular methods identify some toxigenic *C. difficile* that does not necessarily cause infection, others have found that when using clinical diagnosis as reference, PCR accurately detects CDI in a timely fashion and correlates well with clinical diagnosis when compared to culture, cytotoxin neutralization assay and GDH. [17, 18] Careful pre-test patient selection is important to minimize the likelihood of overdiagnosis. One study also found that PCR contributes to increased detection rates of CDI and CDI outbreaks compared to EIA.[19]

The Biofire FilmArray GI Panel is highly sensitive and specific for the 22 microbial targets that it detects. Spina et al. conducted a multicenter, cross-sectional quarterly point-prevalence study of CA-diarrhea in which the majority of samples were received from community-based healthcare providers. Toxigenic *C. difficile* was among some of the most commonly detected enteric pathogens in most participating countries. The FilmArray performed better in detection of *C. difficile* compared to local laboratory protocols.[20] The same conclusions were reached by a multicenter evaluation of the FilmArray done in the U.S., and the detection of *C. difficile* was higher with the FilmArray than with a real-time PCR assay and sequence analysis that was used as a comparator. *C. difficile* was again among the most common enteric pathogens detected.[21]

Although relatively rare in healthy adults, asymptomatic *C difficile* colonization, even with toxigenic strains, is common in neonates and children less than two years of age. Asymptomatic colonization of *C. difficile* can confound diagnostic testing, especially with highly sensitive molecular assays. The American Academy of Pediatrics does not recommend routine testing for *Clostridium difficile* in children, and positive results in children under 2 years of age should be interpreted cautiously.[4, 21]

Institutions should use an algorithm for CDI diagnosis that best fits their needs. Some institutions that readily use NAAT remain wary about the clinical significance of a positive *C. difficile* result and whether this warrants treatment. In some institutions this has even led to the suppression of *C. difficile* toxin NAAT results on the FilmArray GI panel.[22] Some institutions have determined that PCR testing alone is not adequate to identify patients with CDI requiring treatment and that suppressing reports of *Clostridium difficile* detection facilitates antimicrobial stewardship.[23] However it is important to recognize that this may also result in underdiagnosis

and delayed treatment of CDI. It is well established that repeat testing, especially for test of cure, is not recommended when NAATs are used for determination of CDI, which is why institutions often limit follow up testing for a determined amount of time. At UWMC and HMC, repeat testing after a positive result for *Clostridium difficile* is not allowed for fourteen days when ordering direct *C. difficile* testing by GeneXpert. At these same institutions, the detection of *C. difficile tcdA/tcdB* is reported from the FilmArray. As previously mentioned, FilmArray comprehensively tests for an array of different enteric pathogens that could be the cause of diarrhea, and not exclusively for *C. difficile.*

4. Methods

4.1 Cepheid GeneXpert *C. difficile*/Epi Assay

C. difficile testing for the GeneXpert is performed on raw stool received in a sterile container that does not contain any type of preservative. Consistency of the stool can range from watery to soft stool that takes the form of the container. Formed stool is not an appropriate specimen and rejected, as such patients are unlikely to have CDI. A swab is lightly dipped into the stool specimen and placed into the vial of sample reagent and broken off. The sample reagent is vortexed at high speed for ten seconds. Using a sterile transfer pipette, the whole content of the sample reagent is transferred to the test cartridge. The lid of the cartridge is closed and placed into one of the instrument modules. The instrument proceeds to conduct sample purification, followed by nucleic acid amplification and detection of the toxin B gene (*tcdB*) using real-time PCR. The results are interpolated from measured fluorescent signals and embedded calculation algorithms within the GeneXpert Dx System.

4.2 Biofire FilmArray Gastrointestinal (GI) Panel

Testing for the FilmArray GI Panel is performed on stool preserved in Cary-Blair medium. If raw stool is collected, it must be placed into Cary-Blair medium within two hours if transport to the lab is delayed. The FilmArray pouch is prepared by first injecting a hydration solution into the wells containing the freeze dried reagents used for the assay. A sample mix is prepared by adding 200 µl of the stool in the Cary-Blair medium to a sample injection vial containing sample buffer. The vial is mixed by gentle inversion and then injected into the pouch filling the sample well. The pouch is then placed into the instrument. The instrument begins the process with nucleic acid purification followed by reverse transcription for the RNA viruses in the panel and the first stage multiplex PCR. The products of the first stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye which is distributed over the second PCR array. The individual wells contain primers for different assays that target specific nucleic acid sequences for each pathogen on the panel. The panel contains targets for both the toxin A (*tcdA*) and toxin B (*tcdB*) genes. Detection of the targets after PCR is done by DNA melting analysis. Fluorescence is monitored at a certain temperature range, and the software analyzes the melting curve produced and then interprets and reports the result.

4.3 Data Collection

Data were procured from the laboratory information system (LIS) by first obtaining all test accessions with corresponding medical record numbers (MRN) when searching for *C. difficile*-positive results from January and February 2016 under the Cepheid GeneXpert test battery. Test accessions and the corresponding MRNs were then uploaded from January and February 2017 for any *C. difficile*-positive results under the GI panel performed on the Biofire FilmArray.

Next, using the EPIC and ORCA electronic medical record (EMR) systems, patient information relevant to this study was gathered. Location was either determined to be inpatient or outpatient. Stool samples from patients who were experiencing onset of diarrhea in the community that were collected and sent to the lab from outpatient clinics as well as from the UWMC and HMC emergency departments were considered "outpatient". Stools that were collected and submitted to the lab from patients experiencing the onset of diarrhea during their admission at either hospital were considered "inpatient".

One positive *C. difficile* result per patient was allowed within the time period studied. Any repeat positive results were excluded. Positive results from any patient less than two years of age were excluded, since the presence of *C. difficile*, even toxigenic strains, in that age group is more likely to be due to colonization than active infection. Patient charts varied in information given, and charts missing information for all relevant queries were also excluded.

Information from the patient chart included demographics, age and sex, symptoms noted at the time of presentation including fever (>38°C), headache, abdominal pain/cramping, tenesmus or rectal cramping, nausea, vomiting, and diarrhea, and whether the consistency of diarrhea was watery and/or containing blood.

Risk factors that were considered included exposure to antibiotics, proton pump inhibitors, immunosuppressants and recent inpatient stay. Antibiotics were further stratified by the last time the antibiotic was taken. Times considered were within seven days of presentation, more than seven days but thirty days or less, or greater than thirty days. Recent history of an inpatient stay was considered and stratified as less than four weeks, four to twelve weeks, or greater than twelve weeks.

4.4 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, Inc. La Jolla, CA). Student's t-test was used to determine significance among continuous variables, and Chi-square was used for categorical variables. Adjustment for the multiple variables tested was made by Bonferroni's correction.

5. Results

One-hundred fifty seven *C. difficile* results were found searching through the LIS during the months of January and February in 2016 and 2017. One-hundred twenty eight in 2016 were found when searching for positive results from the Cepheid GeneXpert assay. Twenty-nine in 2017 were found when searching for positive results from the Biofire FilmArray assay. In the 2016 portion of the study, 8 repeat positives and 7 cases whose patient charts were incomplete in all areas were excluded. There were 2 cases excluded from the 2017 sample: 1 incomplete chart, and 1 child who was 1 month of age. One-hundred forty were further investigated.

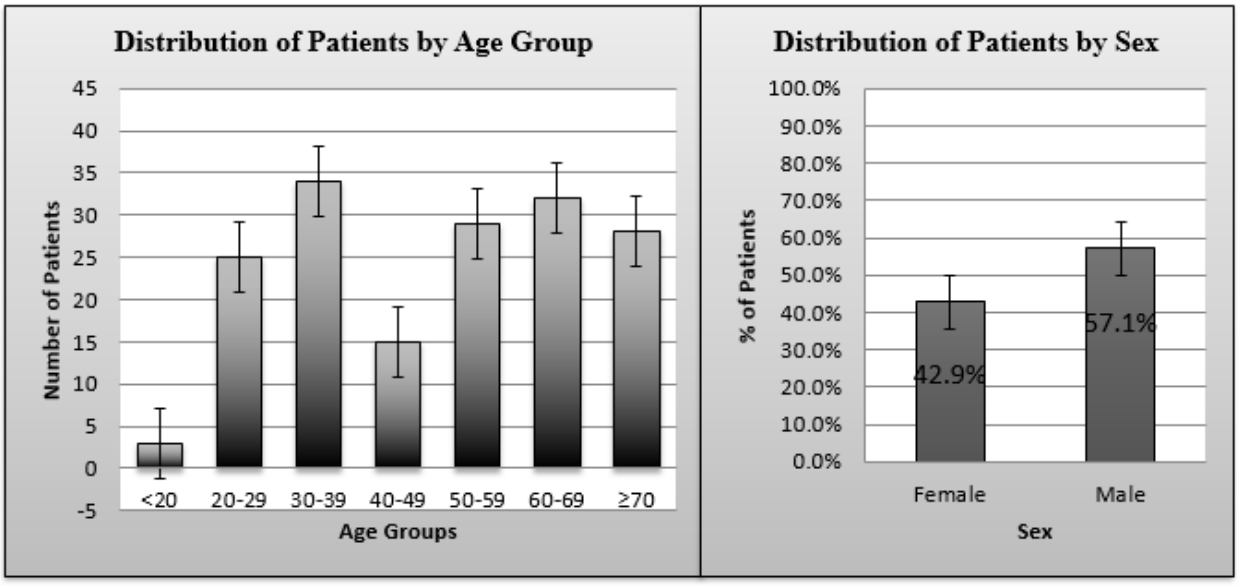
First, the patient demographics of age and sex, the continuous variables, were reviewed. Symptoms and risk factors were categorical variables that were extracted from the patient charts. As antibiotic exposure is a primary risk factor for CDI, the type of antibiotic the patient was currently using or had used prior to the onset of symptoms was recorded. Other information gathered from the patient charts were any comorbidities that the patient had concurrently been experiencing at the time of presentation. Another important risk factor is a history of an inpatient stay, which was used to indicate whether cases were hospital- or community-acquired. The final portion of the analysis focused on the Biofire FilmArray's ability to detect significant cases of CA-CDI. The patient chart was examined for treatment and outcomes of a positive *C. difficile*

toxin A/B result. This included determining wither CDI was part of the differential diagnosis, which antibiotic treatment was used, and whether treatment was successful.

5.1 Age and Sex

The age distribution for the study ranged from 17 to 100 years of age, and the female-to-male ratio favored males in this sample (Figure 3). The age distribution was approximately the same for both the 2016 and 2017 study periods, with more cases at advancing ages. Age and sex were stratified by inpatient and outpatient status for the different years of the study (Figure 4). Though not significant by student's t-test, a slight trend toward younger ages in outpatients with CDI was observed. This comparison was made for overall cases that were inpatient or outpatient and for both female and male cases for both time periods.

Figure 3. Overall Distribution of Patients by Age and Sex.



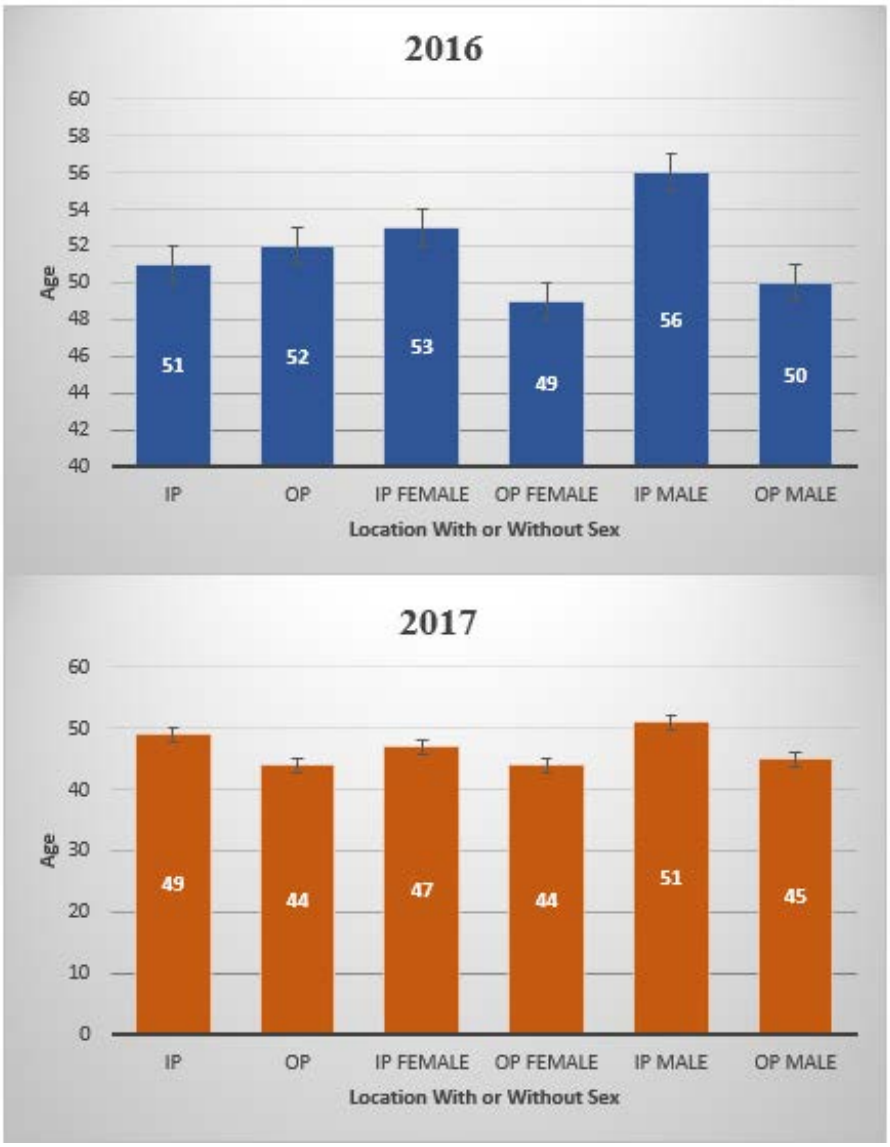


Figure 4. Inpatient and Outpatient Age and Sex.

CDI distribution by inpatient versus outpatient each year. Data labels on the bar are the mean ages for each group. IP = Inpatient OP = Outpatient.

5.2 Symptoms

Three different analyses were performed when comparing groups and symptoms. The first two analyses compared inpatient to outpatient for each time period and the corresponding molecular assay. In 2016 the assay was Cepheid GeneXpert, and in 2017 the Biofire FilmArray. The third comparison was the symptom presentation of the group from the first analysis tested by the Cepheid GeneXpert versus the group tested by Biofire FilmArray (Tables 1-3).

The patients showed no significant differences with regard to most symptoms investigated. In the Cepheid group, there was a significantly higher frequency of abdominal pain ($p = 0.001$) and diarrhea ($p = 0.002$) in outpatient cases (54.2% and 91.5%, respectively) than in inpatient cases (24.1% and 77.8%, respectively). When interpreting Tables 1-3, the addition of Bonferroni's correction lowered the level of significance to $\alpha = 0.005$. Overall, the clinical presentations of inpatients and outpatients with CDI were very similar.

Symptom	Total (n = 113)	Inpatient (n = 54)	Outpatient (n = 59)	P Value*
Fever (>38°C)	15 (13.3%)	7 (13.0%)	8 (13.6%)	0.926
Headache	3 (2.7%)	2 (3.7%)	1 (1.7%)	0.507
Abdominal Pain	45 (39.8%)	13 (24.1%)	32 (54.2%)	**0.001**
Tenesmus	5 (4.4%)	0 (0%)	5 (8.5%)	0.029
Nausea	33 (29.2%)	17 (31.5%)	16 (27.1%)	0.610
Vomiting	19 (16.8%)	10 (18.5%)	9 (15.3%)	0.643
Diarrhea	96 (85.0%)	42 (77.8%)	54 (91.5%)	**0.002**
Watery Diarrhea	44 (38.9%)	17 (31.5%)	27 (45.8%)	0.120
Bloody Diarrhea	6 (5.3%)	0 (0%)	6 (10.2%)	0.016

Table 1. Symptoms Observed in 2016 (Cepheid GeneXpert).

Symptoms as described in patient charts in inpatient and outpatient cases.

*Significance with correction at $\alpha = 0.005$

Symptom	Total (n = 27)	Inpatient (n = 10)	Outpatient (n = 17)	P Value*
Fever (>38°C)	3 (11.1%)	3 (30.0%)	0 (0%)	0.017
Headache	0 (0%)	0 (0%)	0 (0%)	N/A
Abdominal Pain	18 (66.7%)	8 (80.0%)	10 (58.8%)	0.260
Tenesmus	3 (11.1%)	0 (0%)	3 (17.6%)	0.159
Nausea	15 (55.6%)	8 (80.0%)	7 (41.2%)	0.050
Vomiting	10 (37.0%)	5 (50.0%)	5 (29.4%)	0.284
Diarrhea	26 (96.3%)	10 (100.0%)	16 (94.1%)	0.435
Watery Diarrhea	15 (55.6%)	5 (50.0%)	10 (58.8%)	0.666
Bloody Diarrhea	1 (3.7%)	0 (0%)	1 (5.9%)	0.435

Table 2. Symptoms Observed in 2017 (Biofire FilmArray).

Symptoms as described in patient charts in inpatient and outpatient cases.

*Significance with correction at $\alpha = 0.005$

Symptom	Total (n =140)	GeneXpert (n = 113)	FilmArray (n = 27)	P Value*
Fever (>38°C)	18 (12.9%)	15 (13.3%)	3 (11.1%)	0.763
Headache	3 (2.1%)	3 (2.7%)	0 (0%)	0.392
Abdominal Pain	63 (45%)	45 (39.8%)	18 (66.7%)	0.012
Tenesmus	8 (5.7%)	5 (4.4%)	3 (11.1%)	0.179
Nausea	48 (34.3%)	33 (29.2%)	15 (55.6%)	0.010
Vomiting	29 (20.7%)	19 (16.8%)	10 (37.0%)	0.020
Diarrhea	122 (87.1%)	96 (85.0%)	26 (96.3%)	0.114
Watery Diarrhea	59 (42.1%)	44 (38.9%)	15 (55.6%)	0.116
Bloody Diarrhea	7 (5.0%)	6 (5.3%)	1 (3.7%)	0.731

Table 3. Symptoms Observed Between Assay Type Ordered.

* Significance with correction at $\alpha = 0.005$

5.3 Risk Factors

Antimicrobial exposure, PPI use, immunosuppressant drug use and a history of an inpatient stay were evaluated. Comparisons were made between inpatient and outpatient cases in the time periods examined, and between the two molecular assays used (Tables 4-6). All comparisons showed similar risk factor rates, with the exception of exposure to immunosuppressants in the 2016 sample, which was more frequently observed in outpatient cases (49.2%) than in inpatient cases (25.9%) ($p = 0.009$), although in 2017, inpatients (60.0%) had a higher percentage of immunosuppressant use than outpatients (11.8%) ($p = 0.008$). When interpreting Tables 4-6, the addition of Bonferroni's correction lowered the level of significance to $\alpha = 0.013$. This value differs from symptoms because fewer comparisons were made. Immunosuppressant use was not significantly different when comparing the samples tested on the GeneXpert and the FilmArray. In general, patients with CDI in inpatient and outpatient settings appear to be similar with regard to risk factors.

Symptom	Total (n = 113)	Inpatient (n = 54)	Outpatient (n = 59)	P Value*
Antibiotics	70 (61.9%)	32 (59.3%)	37 (62.7%)	0.539
PPI	44 (38.9%)	20 (37.0%)	24 (40.7%)	0.638
Immunosuppressant	43 (38.1%)	14 (25.9%)	29 (49.2%)	**0.009**
Inpatient History	67 (59.3%)	33 (61.1%)	34 (57.6%)	0.589

Table 4. Risk Factors in 2016 (Cepheid GeneXpert).

* Significance with correction is considered at $\alpha = 0.013$

Symptom	Total (n = 27)	Inpatient (n = 10)	Outpatient (n = 17)	P Value*
Antibiotics	18 (66.7%)	7 (70.0%)	11 (64.7%)	0.778
PPI	10 (37.0%)	6 (60.0%)	4 (23.5%)	0.058
Immunosuppressant	8 (29.6%)	6 (60.0%)	2 (11.8%)	**0.008**
Inpatient History	11 (40.7%)	7 (70.0%)	4 (23.5%)	0.018

Table 5. Risk Factors in 2017 (Biofire FilmArray).

* Significance with correction is considered at $\alpha = 0.013$

Symptom	Total (n = 140)	GeneXpert (n = 113)	FilmArray (n = 27)	P Value*
Antibiotics	88 (62.9%)	70 (61.9%)	18 (66.7%)	0.648
PPI	54 (38.6%)	44 (38.9%)	10 (37.0%)	0.855
Immunosuppressant	51 (36.4%)	43 (38.1%)	8 (29.6%)	0.414
Inpatient History	78(55.7%)	67 (59.3%)	11 (40.7%)	0.081

Table 6. Risk Factors Between Assay Type Ordered.

* Significance with correction is considered at $\alpha = 0.013$

5.4 Antibiotic Exposure

Antibiotic exposure is the leading risk factor for acquiring CDI, especially if antibiotics are currently or recently used. As shown in Tables 7 and 8, my observations confirm this association. The vast majority of cases had a history of antibiotic exposure documented in the chart within one week prior to the onset of symptoms. This was more evident for inpatients than for outpatients. Higher proportions of outpatients had prior exposure one month or greater than one month in comparison to inpatients. Traditionally, antibiotic exposure is defined as the use of an antibiotic within 90 days prior to the onset of symptoms. Two outpatient cases from the 2016 sample had prior antibiotic exposure greater than 90 days earlier.

Antibiotic Last Taken	≤ 1 week	> 1 week ≤ 1 month	> 1 month	Unknown
Inpatient n = 32 (%)	27 (84.4)	1 (3.1)	1 (3.1)	3 (9.4)
Outpatient n = 37 (%)	23 (62.2)	5 (13.5)	6 (16.2)†	3 (8.1)

Table 7. Previous Antibiotic Exposure 2016 (Cepheid GeneXpert).
† 2 outpatients in this sample had antibiotic exposure greater than 90 days.

Antibiotic Last Taken	≤ 1 week	> 1 week ≤ 1 month	> 1 month	Unknown
Inpatient n = 7 (%)	5 (71.4)	0 (0.0)	0 (0.0)	2 (28.6)
Outpatient n = 11 (%)	1 (9.1)	7 (63.6)	1 (9.1)	2 (18.2)

Table 8. Previous Antibiotic Exposure 2017 (Biofire FilmArray).

Antibiotic Class	Inpatient n = 39 (%)	Outpatient n = 48 (%)	Total n = 87
Cephalosporins	15 (38.5)	9 (18.8)	24 (27.6)
Glycopeptides	13 (33.3)	9 (18.8)	22 (25.3)
Fluoroquinolones	9 (23.1)	10 (20.8)	19 (21.8)
β-lactamase Inhibitors	14 (35.9)	4 (8.3)	18 (20.7)
Folate Pathway Inhibitors	5 (12.8)	10 (20.8)	15 (17.2)
Carbapenems	7(17.9	3 (6.3)	10 (11.5)
Nitroimidazoles†	3 (7.7)	5 (10.4)	8 (9.2)
Penicillins	1 (2.6)	5 (10.4)	6 (6.9)
Macrolides	4 (10.3)	2 (4.2)	6 (6.9)
Lincosamides‡	1 (2.6)	4 (8.3)	5 (5.7)
Lipopeptides	2 (5.1)	0 (0.0)	2 (2.3)
Oxazolidinones⏚	1 (2.6)	1 (2.1)	2 (2.3)
Tetracyclines	1 (2.6)	0 (0.0)	1 (1.1)

Table 9. Antibiotic Exposure by Class of Antibiotic.
†Metronidazole ‡Clindamycin ⏚Linezolid

Antibiotics were further examined by class (Table 9). Eighty-seven patients had antibiotic exposure throughout both study periods. Thirty-nine were inpatient and 48 were outpatient. The most common antibiotics received by inpatients were cephalosporins (38.5%), whereas outpatients often received fluoroquinolones (20.8%) or the folate pathway inhibitor trimethoprim/sulfamethoxazole (20.8%). The most commonly prescribed antibiotics overall were cephalosporins, fluoroquinolones, and β-lactamase inhibitors, but glycopeptides such as vancomycin were also received by some patients. Glycopeptides were more frequently administered to the inpatient population (33.3%) compared to outpatients (18.8%). Nitroimidazoles, an antibiotic class which includes metronidazole, were also received by some patients (9.2%) prior to the onset of CDI. Although the first line antibiotic treatment for CDI is either metronidazole or vancomycin, 26 cases had previous exposure to vancomycin, metronidazole, or both prior to the onset of their CDI symptoms. However, 11 of these cases had a history of CDI prior to the positive result detected during this study.

5.5 Comorbidities

Patients who have an underlying condition or illness are subject to an increased risk of CDI, so patients were examined for comorbidities (Table 10). Some of these comorbidities are highly related to the risk of CDI. For example, the use of chemotherapy along with other immunosuppressant drugs is seen with patients with underlying malignancies, or those who are afflicted with a form of inflammatory bowel disease, such as Crohn's Disease. Prolonged use of PPIs is observed in patients with chronic GERD. As always, the use of antibiotics are a prime risk factor for CDI. These all are seen in the current study's patient population, along with hypertension, which topped the list of comorbidities.

Comorbidities	Inpatient n=64 (%)	Outpatient n=76 (%)	Total n=140 (%)
Hypertension	22 (34.4)	17 (22.4)	39 (27.9)
Malignancy	13 (20.3)	21 (27.6)	34 (24.3)
Diabetes	18 (28.1)	16 (21.1)	34 (17.1)
IBD	8 (12.5)	16 (21.1)	24 (17.1)
GERD	11 (17.2)	13 (17.1)	24 (17.1)
Infection	10 (15.6)	10 (13.2)	20 (14.3)
Cardiovascular	12 (18.8)	4 (5.3)	16 (11.4)
Trauma	12 (18.8)	2 (2.6)	14 (10.0)
Mental Illness	7 (10.9)	7 (9.2)	14 (10.0)
Substance Abuse	8 (12.5)	5 (6.6)	13 (9.3)
CKD	8 (12.5)	4 (5.3)	12 (8.6)
Hepatitis	5 (7.8)	5 (6.6)	10 (7.1)

Table 10. Comorbidities in *Clostridium difficile* Positive Patients Charts.

The top twelve comorbidities listed in patient charts with positive *Clostridium difficile* results. Inflammatory Bowel Disease (IBD), Gastroesophageal Reflux Disease (GERD), Chronic Kidney Disease (CKD).

5.6 CDI Categorization from Inpatient History

The patient cases that had a previous inpatient history were further stratified by time since inpatient episode and by whether the CDI was hospital- or community-acquired. The general categorization of CDI was as follows:

- If the last inpatient encounter was less than 4 weeks from the time of symptom onset, CDI is considered to be hospital acquired (HA-CDI). Some studies divide this into whether the onset of symptoms was also in a healthcare facility or in the community, but this was not done for this study.
- If the last inpatient encounter was between 4 and 12 weeks from the time of symptom onset, CDI is considered to be indeterminate (I-CDI).

- If the last inpatient encounter was greater than 12 weeks from the onset of symptoms, the case is considered to be community acquired (CA-CDI).

Last Inpatient Encounter	Category	Inpatient n = 40 (%)	Outpatient n = 38 (%)
< 4 weeks	HA-CDI	23 (57.5)	18 (47.4)
4 – 12 weeks	I-CDI	12 (30.0)	6 (15.8)
>12 weeks	CA-CDI	5 (12.5)	14 (36.8)

Table 11. Patients with Inpatient History.

Last Inpatient Encounter	Category	GeneXpert n = 67 (%)	FilmArray n = 11 (%)
< 4 weeks	HA-CDI	37 (55.2%)	4 (36.4%)
4 – 12 weeks	I-CDI	13 (19.4%)	5 (45.5%)
>12 weeks	CA-CDI	17 (25.4%))	2 (18.2%)

Table 12. Inpatient History by Assay Type.

Tables 11 and 12 categorize cases with a history of an inpatient stay as inpatients or outpatients on the basis of the time of diagnosis. The category of HA-CDI included both inpatients and outpatients (57.5% inpatients, 47.4% outpatients). As time elapses since an inpatient encounter, the CDI category changes, which may be indicative of CDI acquisition in the community. Cases observed twelve weeks after an inpatient stay are categorized as CA-CDI. CA-CDI was found in 36.8% of outpatients, which was higher than in inpatients (12.5%).

5.7 Biofire FilmArray Detection of CA-CDI

The FilmArray is a panel of 22 enteric pathogens as opposed to a stand-alone test for the detection of toxigenic *C. difficile*. This study assessed whether the Biofire FilmArray can detect clinically significant cases of CDI, especially CA-CDI. Figure 3 illustrates CA-CDI detection

using the FilmArray. *C. difficile* had to be mentioned in the clinician notes or as a specific ordered test at the time of presentation to be considered as part of the differential diagnosis. The outcomes of treatment were classified as follows:

- Resolved = symptoms abated by the time a single course of the prescribed antibiotic was finished (≤10 days).
- Persistent Symptoms = Symptoms did not resolve within 1 antibiotic course of the antibiotic first prescribed or more than one course was given (>10 days).
- Improved = Symptoms got better by the end of the treatment course but not enough information was provided to know the final outcome of treatment.

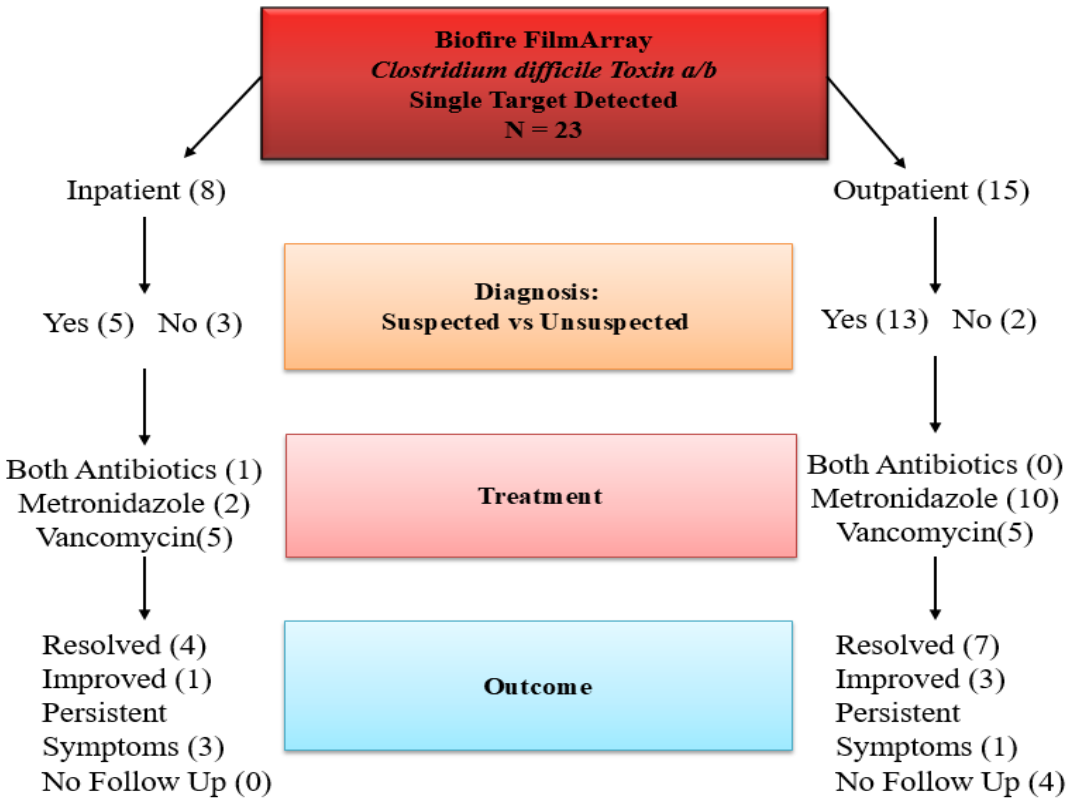


Figure 3. Outcome & Treatment When Positive Only for Biofire FilmArray *C. difficile toxin A/B* Target

Twenty-three patients tested positive solely for *C. difficile* by the FilmArray in the 2017 cohort. This included 8 inpatients and 15 outpatients. These cases were further investigated according to whether *C. difficile* was considered in the differential diagnosis. The inpatients who had unsuspected CDI and had no prior history of CDI were all recently admitted to a hospital. Two had been admitted <24 hours before obtaining a positive result, while one was <72 hours. One patient had recent inpatient exposure less than 4 weeks earlier, another 4 to 12 weeks earlier, and one had no previous exposure, demonstrating that the FilmArray was able to detect unsuspected cases of CA-CDI. Another CA-CDI case was detected in the outpatient cohort. That patient's last inpatient encounter was greater than twelve weeks earlier, although the clinician considered CDI in the differential diagnosis and also ordered GeneXpert *C. difficile* testing, which also was positive. All patients received treatment for CDI. In the inpatient group, 5 received vancomycin, 2 metronidazole, and 1 received both. Treatment began with metronidazole for 8 days, overlapped with vancomycin for 4 days, and then concluded with vancomycin alone for another 4 days. That particular patient was included as an extended treatment outcome. Overall, clinicians appeared to find *C. difficile* positive results clinically significant, and treatment was given to every patient in this group, whether inpatient or outpatient.

6. Discussion

In recent years, the importance of CDI in the community setting has been increasingly appreciated. This shift in the epidemiology of CDI has led to studies comparing symptom and risk factor profiles of HA-CDI and CA-CDI. The laboratory diagnosis for toxigenic *C. difficile* is also evolving, with a greater reliance on PCR based assays, such as Cepheid GeneXpert, that can rapidly and sensitively detected the presence of toxigenic *C. difficile*. The Biofire FilmArray, a

multiplex PCR that detects many different enteric pathogens, includes *C. difficile* as a target. However questions have been raised about the specificity of *C. difficile* detection by a multiplex panel when CDI was not clinically suspected. This study compared cases of CDI detected by FilmArray with those detected by GeneXpert to determine the clinical significance of these assay results.

6.1 CDI Symptoms and Risk Factors

This study made two different comparisons when examining symptoms and risk factors for CDI. First we compared symptoms of inpatients versus outpatients, and found few significant differences when comparing the two groups. In the 2016 sample, abdominal pain and diarrhea were more common in outpatients than inpatients. CDI usually is considered when there are three or more episodes of diarrhea in a 24 hour period. In inpatient scenarios, testing was not always done on watery diarrheal stools, but also with the onset of soft stools. These patients were often being treated with antibiotics and were closely monitored by healthcare workers for changes in stool patterns or consistency. We also compared symptoms between the cases tested on the Cepheid GeneXpert and the Biofire FilmArray and found no clinically significant differences in symptoms exhibited by these two groups.

The same comparisons, of inpatients versus outpatients and GeneXpert versus FilmArray, were done when considering risk factors. There were no significant differences between cases detected by the different molecular assays. Immunosuppressive drugs were significantly associated with outpatient cases in 2016 but with inpatients in 2017, which could be due to a larger number of outpatient cancer-related cases in 2016 (7 out of 29), which were not present in the 2017 sample.

Exposure to antibiotics was the most prevalent risk factor for CDI in our study. About two-thirds of the cases had recent antibiotic use. A large majority of these cases were patients currently on antibiotics or those finished within a week, especially for inpatient cases. The most common antibiotics used were cephalosporins, glycopeptides, fluoroquinolones, β-lactam/β-lactamase inhibitors (especially in inpatients), and folate pathway inhibitors (especially in outpatients). This finding is similar to what is reported in the literature. Meta-analyses to determine the antibiotics that pose the greatest risk for CA-CDI found that clindamycin was most often associated, followed by fluoroquinolones, cephalosporins, penicillins, macrolides, and sulfonamides/trimethoprim.[25,26] On a smaller scale, one hospital looked at the relationship between antibiotics and risk of CDI and found that β-lactam/β-lactamase inhibitors, fluoroquinolones, and cephalosporins were most common. That study did not test for association with CA-CDI.[28] In another study, the same drugs were found in about one-quarter of patients given antibiotics within twelve weeks of presenting with CA-CDI.[29] It was somewhat surprising that, as shown in Table 9, vancomycin and seven metronidazole were also associated, as these drugs are used to treat CDI. However, previous CDI treatment with antibiotics can be a risk factor for recurrent cases, which might explain some of these cases. Thirty-three patients in the overall sample of one-hundred forty had a history of CDI prior to the cases analyzed in the study period.

Comorbidities play an important role in CDI acquisition and often coincide with risk factors associated with CDI. This was confirmed in our study. Many of these conditions are chronic and lead to repeated healthcare encounters, both inpatient and outpatient. Immunosuppressive drugs are used for patients with underlying malignancies, or conditions such as IBD. Antibiotic use could also be tied to other comorbidities like diabetes, hypertension, and

CKD. GERD is often treated with PPIs along with other gastrointestinal medications. Comorbidities also play a role for those who have CA-CDI and lack traditional risk factors. Similar to our findings, conditions like IBD and malignancies, especially hematologic cancers, have previously been associated with an increased risk of CA-CDI.[15] An observation by Khanna et al in a 2012 population-based epidemiological study found that the highest risk of hospitalization from CA-CDI was seen in patients who were older and had underlying comorbidities, a risk that did not decrease in the absence of antibiotic exposure.[16]

6.2 Detection of CA-CDI on Biofire FilmArray

The 2017 sample of this study had stool specimens that were detected by the Biofire FilmArray. Overall there were 27 stools with the toxin A/B target detected. Four of those were co-infections with other enteric pathogens, leaving 23 with *C. difficile* as the only pathogen detected. All of these positive results were acted upon by the clinicians, including 2 patients who developed CA-CDI since their last inpatient encounter more than twelve weeks earlier, as well as others who had no previous inpatient exposure. The finding that all of these results were clinically actionable indicates that the FilmArray is useful to detect cases of CDI, especially in the community setting. One earlier study compared the FilmArray to its standard of care, which for this institution was the Illumigene *C. difficile* amplification assay. The stools were tested whether ordered or not by physicians and then categorized according to whether *C. difficile* stand-alone testing was ordered, ordered along with a FilmArray panel, or no testing by either method was ordered. When *C. difficile* was not initially considered, 8% of samples were positive for the *C. difficile toxin A/B* target. The authors concluded that physician-specified testing for patients with diarrhea can result in underdiagnosis of CDI, which underscores the importance of syndromic panels.[28] A similar study found that CDI was underdiagnosed and that nearly 13% of

CDI cases would have been missed by relying on clinical suspicion. Important risk factors for clinical underdiagnosis of CDI included cases that were community-acquired and younger people presenting with diarrhea. The authors concluded that *C. difficile* testing should be considered even in the absence of known risk factors.[11]

6.3 Limitations

There are some limitations with this study. First, the sample size, though sufficient to detect large differences, was insufficient to detect more subtle and possibly significant differences between the groups. To improve this, a longer period of observation and larger sample size would be required. Second, a retrospective observational study such as this one is open to potential biases and confounders when relying solely on information in the patient chart. To improve data analysis, a prospective study could be performed to follow patients from the time of presentation to the resolution of infection. In order to capture significant differences in symptoms and risk factors, a prospective study could have a case-control design. Persons diagnosed with CDI, whether inpatient or outpatient, could be compared to those diagnosed with a different cause of diarrhea. This approach could be achieved by comparing cases tested with the Biofire FilmArray. This study design could correct for potential recall bias and inaccurate reporting of signs and symptoms. In the present study, the different charting systems used for inpatients and outpatients might have obscured some of the differences between these groups. Third, when considering healthcare history, some exposures to healthcare facilities such as clinic or dental office settings may have been missed. A fourth limitation is the inclusion of cases with a recent history of CDI, which may have confounded some of the risk factors studied. These recurrent cases could be helpful for understanding recurrent CDI, but excluding these cases might provide a better understanding of the initial presentation of CDI, especially in the community setting.

7. Conclusions

C. difficile is an increasingly urgent threat to public health. Developing an improved understanding of the clinical presentation of CDI both in healthcare facilities and community settings is crucial. This study gives a preliminary indication of the relationship between inpatient and outpatient cases of CDI. The literature describes additional studies that collectively provide a foundation for further investigating the differences between HA-CDI and CA-CDI, and this study both confirms and extends those earlier findings. CA-CDI does not always follow the traditional risk factors associated with CDI. However, when looking at CDI in inpatient and outpatient settings, the symptoms at the time of presentation and the associated risk factors are highly similar. This suggests that CDI should be included in the differential diagnosis of acute diarrhea in all clinical settings, especially since CA-CDI can be clinically nondescript and easily overlooked.

Molecular assays have become an integral tool for the rapid detection of toxigenic *C. difficile* along with other enteric pathogens. The Cepheid GeneXpert *C. difficile* assay has been in use for many years at the UWMC and HMC Microbiology Laboratories, and will continue to be used for stand-alone *C. difficile* testing. However the introduction of the Biofire FilmArray provides a new opportunity to detect additional cases of CDI, especially CA-CDI. There is some concern that this method, because of its sensitivity, could potentially detect non-significant cases of CDI that could lead to unnecessary treatment with antibiotics. This is always a caveat of PCR testing, and the clinician needs to take that into consideration when looking at the whole clinical context of a positive result. However, for the microbiology laboratory, it would not be potentially harmful to the patient and misleading to the clinician to suppress the reporting of toxigenic *Clostridium difficile* detection by the FilmArray. This is especially important in cases of CA-

CDI, since this diagnosis may not be initially considered in a clinician's differential diagnosis. Our observations suggest that the detection of *Clostridium difficile* in this setting is often clinically significant.

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