CLOSTRIDIUM DIFFICILE INFECTION SPREAD WITHIN THE HOSPITAL ENVIRONMENT

by

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ABSTRACT

Background: Clostridium difficile Infection (CDI) is one of the leading causes of hospital-associated infections (HAIs) and accounts for nearly half a million infections in the United States (2015). It was associated with approximately 29,000 deaths nationwide in 2011. This study focuses on the role of the environment in the spread of CDI within a hospital in a non-outbreak setting.

Statement of public health significance: The Centers for Disease Control and Prevention (CDC) classifies CDI threat rate as five, which requires urgent and aggressive public health action, because CDI is associated with significant morbidity and mortality. Also, the hospitalization costs associated with CDI increase by over 50%.

Method: This study was conducted at a 495-bed academic University-affiliated single center. The first step includes the performance of bed tracing on all positive CDI admitted patients in the year 2016. The second step included the collection of environmental cultures of the immediate patient surrounding shared devices and floors to identify lapses in environmental cleaning. Aerobic environmental cultures were performed for CDI followed by Gram stain and anaerobic confirmatory culture. Both biochemical and molecular testing was used to confirm Clostridium difficile (CD) presence.
**Results:** Bed tracing was performed for 115 hospital-associated (HA)- and 96 community-associated (CA)- CDI patients. Initial analysis between HA-CDI and CA-CDI revealed that the length of stay was significantly longer in HA-CDIs. However, readmission and recurrence were significantly higher in CA-CDIs. Bed-tracing showed a limited list of high burden rooms. Environmental Cultures revealed only 2 out of 81 surfaces, 14 out of 28 floors & 3 out of 20 wheelchairs as positive for CD spores. None of these patients’ rooms had active CDI patients.

**Conclusion:** Bed tracing and environmental culturing are important public health tools for recognizing rooms with high density of CDI and are particularly important in outbreak settings or any increase in incidence. Shared devices (such as wheelchairs) and floors of patients’ rooms could serve as a reservoir for CD spores. Routine monitoring of disinfection adequacy of shared devices and floors is an important step to assure a safe patient environment.

Lawrence Kingsley, DrPH
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ABBREVIATIONS

CD – Clostridium difficile
CDI – Clostridium difficile infection
HAI – Hospital associated infection
NHSN - National Health Safety Network
EIP - Emerging Infections Program
HA-CDI – Healthcare associated – Clostridium difficile Infection
CA- CDI – Community associated – Clostridium difficile infection
LOS – Length of stay
CDC – Centers for Disease Control and Prevention
US – United States
Tcd A – Toxin A
Tcd B – Toxin B
REA – Restriction enzyme analysis
IRB – Institutional review board
EMR – Electronic medical record
PPI – Proton pump inhibitor
1. INTRODUCTION

*Clostridium difficile* infection (CDI) is one of the leading causes of hospital-associated infections (HAIs). Any individual infected with *Clostridium difficile* (CD) can shed it in feces and any materials or surfaces that come in contact with the feces can act as a reservoir for CD spores (1). These spores persist on surfaces for long periods and can be transmitted to patients in the hospital when they encounter contaminated materials or surfaces (1). To further understand the spread of CDI within the hospital, our research focused on answering the possible role of the hospital environment in the spread of CDI. To study this relationship, we concentrated on evaluating the burden of CD spores in the immediate patient environment and determining the relationship between hospital rooms and CD acquisition.

1.1. Public Health and HAIs

HAIs can be defined as the acquisition of an infection 48 hours after hospital admission or within 30 days of discharge (2). They are infections secondary to the condition a patient is originally admitted for (3). HAIs accounts for nearly 2 million cases and 90,000 deaths annually in the United States, and they are preventable (3). HAIs are also one of the five leading causes of death in acute care hospitals (3). More than 70% of the bacteria causing HAIs are resistant to at least one frequently used drug, contributing to the problem of multi-drug resistant organisms (3). Studies suggest that improving public health surveillance may lead to improved medical procedures and infection control practices, which could lead to a decrease in HAIs (3).
Laws that require reporting of HAI rates were adopted on Aug 27, 2007 by 24 states, which includes Pennsylvania (3). Most often, the Department of Health of each state acts as a regulating agency that is responsible for reporting HAIs (3). However, for the state of Pennsylvania, Pennsylvania Health Care Cost Containment Council acts as the regulating agency for reporting HAIs (3).

Additionally, tools, recommendations and programs that offer infection prevention strategies are developed by the Centers for Disease Control and Prevention (CDC), along with other federal health agencies (4). Data from National Health Safety Network (NHSN), Emerging Infections Program (EIP) and HAI prevalence survey data are used by the CDC to prepare progress reports on preventing five of the most common infections, which includes hospital associated CDI (HA-CDI) (4). According to a report published by CDC, the cases of HA-CDI have reduced since 2012, while the community acquired CDI (CA-CDI) cases have increased. However, the cases of HA CDI (83 per 100,000 persons) are still higher than CA-CDI (66 per 100,000 persons) cases (5) (Figure 1).

Figure 1: Changes over time in crude incidence of CA-CDI and HA-CDI among 10 EIP sites, 2012-2015
1.2. Background on CDI

CD is a bacterium that was first described in 1935 and was identified as a human pathogen in 1978 (6). It is an anaerobic toxin producing bacteria that infects the gut when there is disruption of the gut microbiota (7). This primarily occurs due to the use of antibiotics and environmental contamination (7). The bacterial spores that cause the infection are mainly transmitted via the fecal-oral route (7). The spectrum of CDI varies from asymptomatic colonization to severe, life-threatening toxic megacolon (7). When a patient is carrying or infected with CD, large amounts of CD spores are released into the environment (8). This ensures that CD toxins and spores are present in the environment and hence, spreads continually in humans (8). CD spores are resistant to various cleaning products and alcohol sanitizers which results in widespread dissemination of the spores in closed settings (E.g. Health-care facilities) (9) (10). Recurrence of infection is noted often in roughly 35% of the patients after they use antibiotics (11). The chance of multiple recurrence is almost 50% in patients after the first recurrence (11).

CD has emerged as one of the leading causes of hospital- and community- acquired infections in the United States (7). The Centers for Disease Control and Prevention (CDC) has rated CD threat rate as five on a scale of five and requires urgent and aggressive public health action. This is mainly because CDI is one of the leading causes of HAIs, accounting for nearly half a million infections among patients in the United States in 2015 (12). In acute health care settings alone, the estimated cost for CDI for the US health care system is $4.8 billion per year (7). Hospitalization costs are estimated to be $3,669 per patient and are 54% higher in patients with CDI when compared to patients without the infection. Evidence suggests that this may be due to extended length of stay (LOS) in the hospital (13) (14).
1.3. Epidemiology

HA-CDI has seen a tremendous increase in cases since the early 2000s. The CDC has characterized BI/NAP1/027 strain of CD to exhibit elevated level of fluoroquinolone resistance, efficient sporulation, high toxin production and higher mortality compared to other ribotypes, such as 001 or 014 (15) (16). Ribotype 027 was associated with the largest CD epidemic in Quebec (2005) and was responsible for around 2000 fatalities (17). This strain is also associated with many CD outbreaks and is characterized as a hypervirulent strain (17) (8). However, prior to the year 2000, less than 1% of CDI in the United States was attributed to this strain (15) (16).

According to CDC, CDI resulted in the deaths of around 29,000 patients within 30 days of initial diagnosis (7). Of these, 15,000 deaths were attributed directly to CDI (18). More than 80% of CDI deaths in US occur in patients aged above 65 years (18). Recurrence of CDI is common and one out of every five patients with HA-CDI experience recurrence of the infection (18). Asymptomatic CD colonization can be seen in 2%-3% of the healthy individuals, while 10%-25% of the hospitalized patients have asymptomatic colonization (14).

1.4. Microbiology and Pathogenesis

CD is a gram positive, spore forming anaerobic bacillus that can be found colonized in nearly 2-3% of the adult’s gastrointestinal tract (8) (9). The two microbiological factors that contribute to the virulence include: its ability to sporulate and the ability of the spores to remain viable on both biotic and abiotic surfaces for an extended period (9). Toxin A (TcdA) and Toxin
B (TcdB) are responsible for virulence of CD (4). Ribotype 027 releases 16 times more TcdA and 23 times more TcdB when compared to other stains of CD (8).

Germination of CD spores into vegetative cells takes place in the intestine of the susceptible host (9). This is followed by adhesion of vegetative cells to the epithelial cell layer as the cells infiltrate the surrounding mucus layer (9). Structurally and functionally similar bacterial exotoxins, TcdA and TcdB that can facilitate inflammatory response are released after adhesion, during the late log phase and stationary phase of vegetative growth (9). TcdA and TcdB further glucosylate and inactivate members of Rho family of guanosine triphosphatases (Rho GTPases) (15) (7). This disrupts the actin cytoskeleton, cell rounding, inhibition of cell division, and cell death which leads to neutrophilic colitis, colonocyte death, and loss of intestinal barrier function (15) (7).

Figure 2: Pathogenesis of CDI

1.5. Risk Factors

There are several risk factors for CDI, such as length of hospital stay, antibiotic use, advanced age, higher comorbidity index (CI), number of previous episodes, and female gender (15) (19). Among these, antibiotic use remains the leading cause of CDI. The most frequently associated antibiotics include ampicillin, clindamycin, cephalosporins, amoxicillin and fluoroquinolones (15) (19).
Environmental contamination and antibiotic usage are most commonly associated with hospitals and long-term care facilities (15). There has been an increase in the CA-CDI in the recent years, accounting for around one third of the new CDI cases (15). Onset of disease in a person without overnight stay in health care facility within 12 weeks before the infection is described as community acquired (15). However, morbidity and mortality associated with CA-CDI is lower than HA-CDI. (15) Apart from these, chemotherapy, chronic kidney disease, immunodeficiency, organ transplantation, inflammatory bowel disease and exposure to an infected adult and infant carrier are risk factors for CDI (15).

1.6. Symptoms, Diagnosis and Treatment

The most common symptom of CDI is two to three non-bloody, watery stools for one to two days, which can be accompanied with abdominal pain (20). Fever, shock and severe ileus are the more severe symptoms of CDI (20). Toxin enzyme immunoassay (EIA) and/or polymerase chain reaction (PCR) can be used to detect the presence or absence of CD or its toxins (20) (21). However, no test can diagnose if a person has CDI or not (21). Negative test on EIA but positive test by PCR indicates a less severe infection or mortality due to CDI, as compared to a positive test on both EIA and PCR. A positive test by PCR alone is more likely to identify asymptomatic CD carriers (21).

The treatment recommendations by the Infectious Diseases Society of America/ Society for Healthcare Epidemiology of America, 2010 are as shown in Table 1 (21). The treatment is based on whether the infection was mild or severe (21). Mild CDIs can be treated with metronidazole, while severe CDIs are treated with vancomycin or a combination of metronidazole
and vancomycin. Recurrent CDI is treated with vancomycin. Fidaxomicin or Fecal Microbiota transplantation (FMT) has shown success in treatment of recurrent CDI (21).

Table 1: CDI treatment by severity and recurrence

<table>
<thead>
<tr>
<th>Severity/ Recurrence of the Infection</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild to moderate infection</td>
<td>Metronidazole</td>
</tr>
<tr>
<td>Severe infection</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>Severe, complicated or fulminant</td>
<td>Vancomycin (PO) + Metronidazole (IV)</td>
</tr>
<tr>
<td>First recurrence</td>
<td>Based on severity, either Vancomycin, Metronidazole or combination of both</td>
</tr>
<tr>
<td>More than one recurrence</td>
<td>Vancomycin</td>
</tr>
</tbody>
</table>

1.7. HA-CDI

A susceptible host and an adequate exposure to CD are required for CDI to occur. (22) CDI is more common in intensive care unit (ICU) patients than in non-ICU patients because there is an increased necessity to use antibiotics within this population (14) (23). Also, a patient will be at an increased risk for CDI when the prior occupant of the room was CD positive (22) (14). Similarly, a person sharing the room with a positive CDI roommate will be at an increased risk for the infection (22).
1.7.1. Antibiotics and Patient Tracing

Antibiotics can increase the environmental burden of CD by promoting its proliferation and increasing the number of CD spores shed into the environment. This can increase the risk for CD acquisition and infection in patients sharing the same room in future (22). A study conducted in a non-outbreak setting proved that, a bed that was previously occupied by a person who was on antibiotics increased the risk of CDI in subsequent patients, adjusting for confounding factors such as comorbidities, ward type, subsequent patients’ exposure to antibiotics and colonization pressure of CDI (22).

A study conducted in a university-affiliated medical center found a relationship between use of levofloxacin and increase in CDI in an outbreak setting (13). This association was established as levofloxacin was introduced to the hospital’s formulary and there was an increase in the quinoline use prior to the outbreak (13). Also, the study considers clindamycin and ceftriaxone as risk factors. For 87 nosocomial CDI, restriction enzyme analysis (REA) type 2 was traced to case-patients housed on one particular floor and part of the hospital (13 out of 17 patients), whereas type 4 was predominant in another part of the hospital (21 of 26 patients) (13).

1.7.2. CD in Hospital Environment

Rapid acquisition of microorganisms present in the local environment takes place when an individual enters a new environment, which might include CD (22). A study demonstrated that there was a minimum of one pathogenic organism in 39% of the patients’ hands and more than or equal to 2 pathogens in 8% of the patient’s hands, of which 14% were CD positive (24).
CD spores can persist in the environment for up to five months and can be isolated from beds, bed rails, walls and floors of the hospital rooms that were previously occupied by a CDI patient (22) (25) (14). Long survival time further helps in disseminating the CD spores to surfaces beyond the immediate patient environment (25). CD can be cultured from 49% and 29% of the surfaces of hospital rooms that were previously occupied by a CD positive patient and asymptomatic carriers respectively (14). 90% of the floors of bathrooms and corners of the isolation rooms in the hospital can be contaminated by CD. CD is one of the most frequently recovered pathogens from the hospital floor (25) (26). It is possible for some frequently touched objects such as a call button, linens and medical devices to come in contact with the floor and promote the transmission of pathogens to the patients’ hands (26).

The fecal-oral route is suggested to be one of the modes of transmission of CD spores in the hospital (24). It is observed that patients wash their hands less frequently in a hospital. A study conducted on hand hygiene focused on educating the nurses to educate patients to improve the frequency of hand wash before and after certain activities in the hospital. A notable decrease in the number of HA-CDI cases, from 22 in quarter (Q) 1 to 16 in Q2 and 11 in Q3 was observed after the implementation of patients’ hand hygiene (24).

Cleaning the hospital rooms with 1:10 dilution of bleach or sporicidal agents are proven to be effective in reducing the environmental burden of CD (25) (14). CDI spread can be limited if room cleaning is associated with hand washing, contact precautions and isolation of patients upon symptoms (25) (14). These precautions are recommended only for rooms and or patients with positive CDI (25). It is important to note that patients can remain asymptomatic and can contribute to contaminating the hospital environment (25). Studies have shown that decontamination of the
hospital room with hydrogen peroxide vapor could reduce the incidence of HA-CDI (14). However, even after disinfecting the surfaces, CD ribotype 027 can persist for up to 60 min (25).
2. METHODS

The study was conducted at a 495-bed university-affiliated medical center, concentrating on the pattern of spread of CDI within the hospital environment. The study was approved by the University of Pittsburgh Institutional Review Board (IRB) as a Quality Improvement study on April 05, 2017 (Project ID: 1033).

2.1. Research Questions

In this study, we are trying to find an answer to “What is the role of environment in spread of CDI within the hospital environment, in a non-outbreak setting?” To achieve this, we tried to identify the relationship between a hospital room and CD acquisition and identified the burden of CD spores in the immediate patient environment.

Traditional bed-tracing was carried out for all CDI patients in 2016 (regardless of CA- or HA-CDI), new cases as well as history of recent CDI. This was followed by identifying the percentage of patients’ who were tested positive after leaving a room. Environmental culturing of all the ICUs, MICUs, trauma and burn units, rehabilitation units and 20 wheelchairs was performed to identify the prevalence of CD in the patients’ room and immediate patient environment.

2.2. Bed Tracing

Bed tracing in a non-outbreak setting was carried out to identify the pattern of spread of CDI within the hospital environment. To analyze this pattern, the movement of all the CD positive
patients in 2016 were traced. Data collection, data sorting and analysis (details below) were the three main stages in bed tracing.

2.2.1. Data Collection

Data was collected for all the in-patients who were tested positive for CDI in 2016 and their movement within the hospital was traced from Jan 1, 2016 through Jun 15, 2017. More information on each of the CDI patients were obtained from the Electronic Medical Records (EMR; Theradoc). The collected information includes patients’ age, sex, race, 2016 admission- and discharge-date, death date (if applicable), possible HAI or CAI, comorbidities, readmission and the number of times readmitted, note on antibiotics, use of proton pump inhibitors (PPI), CD positive test date, History of CDI, LOS and movement within the hospital (bed tracing). LOS was calculated as the first hospital stay in 2016. We assumed a gap of less than 48 hours as same admission. All the patients were given a subject ID, which was the first letter of their first and the last name followed by the last four digits of their unique identification number.

2.2.2. Data Sorting

The collected data on history of CDI, readmission, infection classification, Charlson’s CI, antibiotics and PPI use were sorted into either two or more categories for data analysis. More description on each of the variables are given below and in Figure 3.
• History of CDI: The history of CDI within two years, 2014 and 2015 were collected and a score of one was given if the patient was tested positive anytime in 2014 or 2015. If the patient was tested positive only in 2016, the score was zero.

• Readmission (31): A patient was considered as readmitted and was given a score of one if they were admitted 90 days prior to their first 2016 CD positive test stay, else they were marked as zero and were not considered for further classification based on readmission. Number of times a patient was readmitted was also considered. A patient was scored as one, if they were readmitted once; score of two if the patient was readmitted twice or more 90 days prior to being tested positive.

• HAI/CAI Classification (2): Most of the patients were classified either as possible/confirmed HAI or CAI. However, a few unclassified patients were manually classified depending on the date of admission and test result of positive CDI. A patient was considered to have HA-CDI if they were tested positive 48 hours after being admitted to the hospital. However, for statistical analysis, only the patients’ confirmed on hospital records were considered.

• Charlson’s comorbidity index (CI) (30): CI was noted for all the patients. CI was considered as zero if the patients did not fall under any of the categories on Charlson’s CI. Along with the total score, a list of all the conditions and their respective scores were mentioned.
- **Antibiotics**: Only the antibiotics used during the hospital stay were considered. If the patients did not use any antibiotics during their stay, they were scored as zero, else one.

- **PPI**: Use of PPI during the hospital stay was considered to be one, else zero.

**Figure 3: Algorithm used for data sorting**

(Blue color indicates the end points; red color indicates the path taken for the study; green color indicates the different categories that were considered; orange color indicates the intermediate steps taken to reach the end-point)
2.2.3. Data Analysis

Statistical analysis was performed using SAS version 9.1. Fishers exact test was used to compare HA-CDI and CA-CDI (outcome variables) with different parameters such as history of CDI, readmission, use of antibiotics and PPIs (exposure variables). All the variables analyzed were categorical variables with two levels. The Kruskal-Wallis test was used to identify the differences in continuous variables such as age, CI and LOS between the HA-CDI and CA-CDI patients. The results were considered to be statistically significant if the P value was \( \leq 0.05 \).

The data from bed tracing was used to identify the rooms that had high density of CD positive patients. The top 13 rooms were further analyzed to classify if the patients were negative or positive while staying in a particular high-density room with the following assumptions:

- Same stay was given more preference
- If a patient was tested positive on multiple dates, then the test closest to the room stay was considered
- If a patient had stayed in a room multiple times during the same stay when being tested positive for CD, then the closest date was considered
- Readmission within 48 hours was considered as same admission
- 2016 admission was given preference. Even when the person was in that room in 2015 (as part of the continued stay to 2016), regardless of the closest date to the 2015 positive test

If a patient had stayed in a room in question prior to being tested positive for CDI, they were considered as negative; if they stayed in a room after or while testing positive for CDI, they were considered as positive; patients with multiple admissions and staying in a room during one
of their stays when they were not tested positive for CDI, was classified as different stay. The corresponding dates on when the patients entered, and exited the room was also noted and were organized in ascending order. The duration between each positive stay was calculated by counting the number of days from the exit date of previous CDI patient’s stay in that room until the entry date of next stay. The average duration between CDI patients’ stay and percentage of negative patients’ in each of the 13 high-density rooms were calculated to further investigate the possible mode of transmission of CDI.

2.3. Environmental Culturing

The aim of environmental culturing was to estimate the prevalence of CD in the immediate patient environment and to verify the efficacy of cleaning protocols in the hospital. Environmental culturing was performed for 53 ICUs, MICUs, and trauma and burn rooms, 28 rehabilitation rooms and 20 wheelchairs. Contact- and droplet- precautions were followed while culturing the rooms and were noted down if it was mentioned for any of the patient rooms. Empty rooms with- or without- bed/s were also noted while culturing.

Hardy Diagnostics C diff Banana (Figure 4) Broth was used to detect CD in the environment. Sterile cotton swabs were moistened with broth and the patients’ immediate environment was cultured. After culturing the rooms, the swabs were inserted into the culture tubes and tightly closed as shown in Figure 5. The broth tubes were then incubated at around 34 degrees Celsius for seven days and were checked for color change every 24 hours. If any of the tubes changed color from red to yellow, confirmatory tests were carried out in the microbiology lab. As part of the confirmatory tests, the positive culture from the broth tube was streaked on blood agar
plates and were incubated at under anaerobic conditions at around 35 degrees Celsius for 24 hours. After which, gram staining (to confirm the morphology); biochemical tests (using the Thermo Fisher readymade biochemical test plates as shown in Figure 6); and PCR were performed.

Figure 4: 10 ml tubes of Hardy Diagnostics C diff Banana Broth

Figure 5: Culture tubes before incubation

Figure 6: RapID Inoculation fluid and ANA II systems for biochemical tests of anaerobic bacteria
The culture sites for ICU’s, MICUs and trauma and burn rooms included bed rails, table, monitors, pumps, vents, tube feeding pump, infusion pump, oxygen fixture, cable insertion to the monitor and call button as shown in Figure 7. All the rooms were single-bed rooms without attached toilets and curtains were substituted for entrance doors and one side of the wall. One tube of Hardy diagnostics C diff banana broth was used per room to swab all the above-mentioned sites. The tubes were labelled as ‘C’ for ICUs, ‘M’ for MICUs and ‘B’ for trauma and burn units before starting room culturing.

Rehabilitation rooms were mostly private-single-bed rooms with attached toilets and had an entrance door with walls. Whereas, a few rooms were semiprivate rooms with two beds, separated from each other by a curtain. Patients’ in semiprivate rooms had a shared toilet. For culturing rehabilitation rooms, two Hardy diagnostics C diff banana broth tubes were used per room, irrespective of private- or semiprivate- rooms: one for the floor, which included corners of the room floor and toilet floor; and another for the surfaces, which included toilet seats, phone, call button, bed rails and tables as shown in Figure 8 and Figure 9. The tubes were labelled as ‘F’ for floor and ‘S’ for surfaces followed by the room number.

Wheelchairs that were cultured were not-currently-in-use by the patients. The cultured sites included the most frequent spots touched by the patients while using a wheelchair and did not
include the wheels. One Hardy diagnostics C diff banana broth tube was used per wheelchair as shown in Figure 10. Each tube was labelled as ‘WC’ followed by the wheelchair number.

Figure 8: Tube 1- corners of the room floor and toilet floor

Figure 9: Tube 2- Toilet seats, phone, call button, bed rails and tables

Figure 10: Tube 1- Wheelchair culturing
3. RESULTS

An average of 1200 tests for CD are conducted annually in this university affiliated medical center. The overall rate of positive tests was 6-9% for unique patient testing. In 2016, 211 patients tested positive for CDI. Of that number, 115 patients were classified as HA-CDI and 96 were classified as CA-CDI.

3.1. Statistical Analysis

The Baseline characteristics such as age, gender, CI, readmission, number of times readmitted, History of CDI, LOS, race, antibiotics and PPI use during the hospital stay for HA- and CA- CDI was compared as shown in Table 2. Table 2 shows the number of patients in each category and the average age and CI for the two groups. The mean age of all the patients in the study was 63 years. There was no significant difference in age between HAI (65 years) and CAI (62 years) groups from Kruskal–Wallis test as shown in Figure 11. Charlson’s CI for the entire cohort was 4 and this was same for both groups. However, there is stark difference in the LOS between the two groups, 25 days for HA-CDI and 9 days for CA-CDI. Overall, the percentage of readmission within 90 days was 15.6%. Of this, 6.6% of the patients were readmitted twice or more prior to being tested positive for CD for the first time in 2016.

Table 3 shows the results from Fisher’s exact test. For statistical analysis, only the HA- or CA- CDI confirmed on EMR were considered. A total of 196 patients were considered, with 85 classified as CA-CDI and 111 as HA-CDI. A statistically significant difference (P-value ≤ 0.05) was observed in three categories, history of CDI, PPI use and readmission. Patients with CA-CDI
(22.2%) were more likely to have recurrence of CDI and readmission when compared to HA-CDI (9%) patients (P value= 0.04). Among patients with HA-CDI, 76.6% used PPIs during their stay at the hospital, while among CA-CDI, it was only 61.2% (P value= 0.03).

Table 2: Baseline characteristics of CDI cases, HA-CDI and CA-CDI

<table>
<thead>
<tr>
<th>Metric</th>
<th>Total +ve C diff cases</th>
<th>CA-CDI</th>
<th>HA-CDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean</td>
<td>63</td>
<td>65</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>95</td>
<td>51</td>
<td>44</td>
</tr>
<tr>
<td>Male</td>
<td>116</td>
<td>45</td>
<td>71</td>
</tr>
<tr>
<td>Co-morbidity Index</td>
<td>Mean</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Readmission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>32</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>No</td>
<td>179</td>
<td>77</td>
<td>102</td>
</tr>
<tr>
<td>No of times re-admitted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>176</td>
<td>75</td>
<td>101</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>2 or more</td>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>205</td>
<td>91</td>
<td>114</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>PPI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>147</td>
<td>52</td>
<td>89</td>
</tr>
<tr>
<td>Negative</td>
<td>64</td>
<td>33</td>
<td>26</td>
</tr>
<tr>
<td>History of CDI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>178</td>
<td>75</td>
<td>103</td>
</tr>
<tr>
<td>1</td>
<td>33</td>
<td>21</td>
<td>12</td>
</tr>
<tr>
<td>LOS</td>
<td>Mean</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>31</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>white</td>
<td>170</td>
<td>72</td>
<td>98</td>
</tr>
<tr>
<td>other</td>
<td>10</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
Table 3: Statistical analysis using Fisher’s Exact test

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HA-CDI (%)</th>
<th>CA-CDI (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of CDI</td>
<td>9.90</td>
<td>21.20</td>
<td>0.04</td>
</tr>
<tr>
<td>PPI use</td>
<td>76.6</td>
<td>61.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Antibiotic use</td>
<td>99.1</td>
<td>96.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Readmission</td>
<td>9.9</td>
<td>21.2</td>
<td>0.04</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>60.4</td>
<td>48.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Female</td>
<td>39.6</td>
<td>51.8</td>
<td></td>
</tr>
<tr>
<td>Race: white</td>
<td>85.6</td>
<td>76.5</td>
<td>0.1</td>
</tr>
<tr>
<td>All-cause mortality</td>
<td>28.8</td>
<td>21.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The results from Kruskal-Wallis test suggests that there was no statistically significant difference in age and Charlson’s CI between HA-CDI and CA-CDI as shown in Figure 11 and Figure 13 respectively. However, there was a significant difference in the LOS, higher in HA-CDI patients as shown in Figure 12.

![Distribution of Wilcoxon Scores for age](image-url)

**Figure 11: Wilcoxon scores for age**
3.2. Bed tracing

Bed-tracing showed specific medical rooms, intensive care unit rooms and rehabilitation rooms with highest CD patient-days. Some of the units with highest number of CDI patients are shown in Table 4. Units U10E, U12E and U9E are medical units with an average of 45 beds per floor; Units U3E1 and U4F2 are ICUs with 53 beds; and U6E, U6F, U7A, U7E and U7F are
rehabilitation rooms with 65 single beds. There were a total of 470 CDI patients who stayed in medical units, irrespective of whether they were tested positive during, before or after the room stay. This was followed by ICUs with 268 patients and rehabilitation units with 162 patients.

<table>
<thead>
<tr>
<th>Units</th>
<th>Total patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>U10E</td>
<td>172</td>
</tr>
<tr>
<td>U12E</td>
<td>155</td>
</tr>
<tr>
<td>U3E1</td>
<td>114</td>
</tr>
<tr>
<td>U4F2</td>
<td>124</td>
</tr>
<tr>
<td>U6E</td>
<td>20</td>
</tr>
<tr>
<td>U6F</td>
<td>48</td>
</tr>
<tr>
<td>U7A</td>
<td>13</td>
</tr>
<tr>
<td>U7E</td>
<td>12</td>
</tr>
<tr>
<td>U7F</td>
<td>69</td>
</tr>
<tr>
<td>U9E</td>
<td>143</td>
</tr>
</tbody>
</table>

Table 4: Total number of CDI positive patients in selected units

With respect to the rooms, UDEM had 156 CDI patients tested in the year 2016 and traced through Jun 15, 2017 as shown in Figure 14. This was followed by room U1E 1010 01 with 59 patients. However, most of the rooms with high number of CDI patients were emergency rooms and were not considered for room analysis. Room analysis of non-emergency rooms showed U12E U12E0 01 with highest number (16 patients) of CDI patients, followed by U10E 10013 01 with 13 patients as shown in Table 5, which contains the list of top 13 non-emergency rooms with high number of CDI patients, irrespective of whether they were tested before, during or after being tested positive for CD. For all the top 13 rooms, the average duration between two CDI patients room stay was ≥ 20 days (Table 5).
Figure 14: Data on number of patients in each room, includes both emergency and non-emergency rooms

Table 5: Top 13 non-emergency rooms

<table>
<thead>
<tr>
<th>Non-emergency rooms</th>
<th>Number of CDI patients</th>
<th>Average duration between CDI patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>U12E U12E0 01</td>
<td>16</td>
<td>28.1</td>
</tr>
<tr>
<td>U10E 10013 01</td>
<td>13</td>
<td>34.5</td>
</tr>
<tr>
<td>U10E 10015 01</td>
<td>12</td>
<td>24.2</td>
</tr>
<tr>
<td>U8E U8E0 01</td>
<td>12</td>
<td>28.5</td>
</tr>
<tr>
<td>U9E 9013 01</td>
<td>12</td>
<td>24.5</td>
</tr>
<tr>
<td>U3E1 3010 05</td>
<td>10</td>
<td>37.8</td>
</tr>
<tr>
<td>U4F2 4223 17</td>
<td>10</td>
<td>30.6</td>
</tr>
<tr>
<td>U7F 7212 01</td>
<td>9</td>
<td>27.9</td>
</tr>
<tr>
<td>U4F2 4223 02</td>
<td>9</td>
<td>38.3</td>
</tr>
<tr>
<td>U4F2 4223 11</td>
<td>9</td>
<td>26.5</td>
</tr>
<tr>
<td>U3E1 3010 06</td>
<td>9</td>
<td>35.1</td>
</tr>
<tr>
<td>U9E U9E0 01</td>
<td>9</td>
<td>36.7</td>
</tr>
<tr>
<td>U4F2 4223 10</td>
<td>9</td>
<td>20</td>
</tr>
</tbody>
</table>

Analyzing the top 13 non-emergency rooms (Figure 15) helped us identify rooms where a person was negative for CDI while staying in that room and later become positive, i.e. the patient became positive for CDI after moving out of the room. For instance, 58.3% of the of the patients
who stayed in U8E U8E0 01 were tested positive after staying in that room. This was followed by U4F2 4223 02 with 44% of patients being tested positive after room stay. However, none of the patients were tested positive after staying in U10E 10013 01. All the patients staying in this room were either already positive or were staying in that room as part of another hospital admission.

Most of the top 13 non-emergency rooms had patients who were tested positive for CD while staying in a room or moved to a room after testing positive. These patients were all considered to be positive. U9E U9E0 01 had almost 79% of positive patients followed by U10E 10013 01, U9E 9013 01, U7F 7212 01 with more than 65% of positive patients.

**Figure 15: Analysis of top 13 non-emergency rooms**
(Blue bars indicate the percentage of patients staying in the room before testing positive for CDI; Orange bars indicate the number of patients staying in the room while- or after- testing positive for CDI; Gray bars indicate the percentage of patients who were tested for CDI in a different hospital admission)
3.3. Environmental Culturing

The change in color from red to yellow of the Hardy Diagnostics C diff banana broth as seen in Figure 16 indicates presence of CD or its spores in the environment. Environmental culturing of medical and intensive care units showed no evidence of CD spores on the surfaces of the immediate patient environment. Whereas, nineteen rehabilitation rooms (two surface tubes and 17 floor tubes) and three wheelchairs have shown positive results upon culturing as shown in Figure 17. Confirmatory test was carried out for all the 19 positive tubes from the rehabilitation rooms. The growth on agar plates were as shown in Figure 18; gram positive rods upon gram staining was observed under the microscope as shown in Figure 19; color change in some specific wells of the biochemical test plates as shown in Figure 20; and positive PCR results were seen in 14 out of the 19 tubes.

From the top-13 rooms, nine of them (U12E U12E0 01, U10E 10013 01, U10E 10015 01, U3E1 3010 05, U4F2 4223 17, U4F2 4223 02, U4F2 4223 11, U3E1 3010 06 and U4F2 4223 10) were cultured. Out of which, only one room (U12E U12E0 01) was tested positive.

Figure 16: Positive result: Change in color of the Hardy Diagnostics C diff banana broth from red to yellow
Figure 17: Positive results from rehabilitation rooms and wheelchairs

Figure 18: Growth of CD on agar plates after 24-hour anaerobic incubation

Figure 19: Gram staining – gram positive rods

Figure 20: Anaerobic biochemical test results
4. FINDINGS

    Bed tracing was helpful in identifying specific rooms with high number of CD patients. The bed tracing results helped us identify certain rooms that could be associated with CDI acquisition. For instance, we found that around 16% of the patients were tested positive for CDI ≤ one week after their room stay in at least one of the high-density rooms. From this we can suspect that the patients could have acquired the infection from these rooms.

    Results from environmental culturing revealed that CD is existing only on the floors of patient rooms and not on surfaces. This is probably because the surfaces are frequently cleaned with sporicidal agents, while the floors are not. This research illustrates the necessity for a policy change in the university affiliated medical center to clean floors with sporicidal agents. Finally, this research showed that wheelchairs are harder to clean and may require specific handling.
5. DISCUSSION

CDI is the leading cause of HAIs worldwide, mostly affecting the elderly population. It is possible to prevent CDI in a healthcare setting by following an effective infection control and antimicrobial stewardship programs. The study conducted at university affiliated single center focused on reducing the HA-CDI by analyzing the pattern of CDI spread within the hospital environment.

The results from our study was consistent with the results from other studies on CDI. In general, around 80% of the CDI patients were over the age of 50 years, 97% of them were using antibiotics during their hospital stay and 70% of the patients were on PPIs. The average Charlson’s CI was four and remained the same among CA-CDIs and HA-CDIs. All the factors mentioned above are considered risk factors for CDI. During the study period, the percentage of all-cause-mortality was around 25%, which was higher in HA-CDI as compared to CA-CDI. However, the results were not statistically significant. History of CDI and/or readmission was seen in 15% of the patients and was significantly higher in CA-CDIs. Females are considered to be at a higher risk for CDI, though our study found the number of males infected with CDI were higher than the number of females.

Previous studies on patient tracing suggest that placement of non-CDI patients in a room that was previously occupied by a CDI patient plays an important role in transmission of the infection (22) (14). In addition, most of these studies are carried out in an outbreak setting. Although, we found bed tracing to be difficult to analyze, we were able to identify the percentage of patients’ who tested negative for CDI in the high-density rooms and later became positive after moving out of the room. In total, 22% of the patients tested positive for CDI after staying in at
least one of the high-density rooms. Out of these, more than 16% of the patients tested positive for CDI ≤ 1 week after their room stay in one of the top-13 rooms. This suggests the patient acquired CDI after staying in the room. However, the case-only study design makes it difficult to draw a definite cause/effect. Furthermore, placement of CDI patients in particular isolation rooms can bias the results. A patient might have stayed in one of the top-13 rooms for a long period of time and probably at the end of the room stay tested positive for CDI. They are still considered under the positive category and are not included in the analysis. This might have confounded our results because the room could have contributed as they had stayed there for a long time.

The causes for the spread of CDI within the hospital environment are still not clearly understood. Around 50% of the room floors were positive for CD on confirmatory tests. These results are consistent with other studies that showed CD as the most commonly recovered pathogen from the floor, regardless of whether it was occupied by a CDI patient or not (26). Studies suggest that CD can be recovered from more than 30% of the surfaces in hospital rooms, however, our study found only 2% of the surfaces positive for CD (14). Even though the specificity of the medium used is 100%, we are unsure of the sensitivity. Studies conducted using a more sensitive sponge swab technique showed 90% of the floors and 74% of the surfaces to be contaminated with CD (25).

The length of stay in ICUs, MICUs, trauma and burn units are lesser than the length of stay in rehabilitation rooms and therefore are cleaned more often. This could be one of the reasons behind none of the cultures showing a positive result. Also, during the time of culture, none of the patients in these units tested positive for CDI. This could be another reason for negative culture results. However, the floors of ICUs, MICUs, trauma and burn units were not cultured because the patients in these rooms were not able to walk and could not have encounter the floor. Whereas,
patients in rehabilitation rooms could walk and it was likely that they had encounter the floor. Also, 15% of the wheelchairs were positive for CD upon culturing. This shows the need to enhance hospital wide cleaning with sporicidal agents.

5.1. Public health and CDI

CDI is traditionally known as an HAI, however, there is an increase in the number of CA-CDI cases in the past decade, especially in patients without any exposure to antibiotics (6 months prior to the study) and of younger age (27). It is difficult to identify the burden of CA-CDI in asymptomatic persons, as screening for CDI is recommended only for symptomatic patients. A study conducted in a tertiary-care medical center focused on screening all the bone marrow transplant patients for CDI at the time of hospital admission. After the implementation of screening intervention, there was a significant reduction in the incidence of HA-CDI (28). Whole-genome sequencing of isolates from patients in a hospital setting showed only 35% of the CDI cases to be genetically related to at least one previous case. This means that the symptomatic patients did not have a major role in the transmission of CDI (29). We can assume that apart from symptomatic and asymptomatic patients, environmental sources such as food, water and animals can act as a reservoir and contribute to the spread of the infection (27).

As discussed before, CDI can cause significant morbidity that can lead to hospitalization, increase the LOS and the hospital expenses, making it an important public health issue. Therefore, appropriate measures should be taken such as environmental cleaning with sporicidal agents and appropriate use of antibiotics should be followed in both community and hospitals.
5.2. Hospitals and healthcare professionals

Healthcare professionals can also play an important role in transmission of CD spores. Hand hygiene (washing of hands with soap and water) protocol should be followed by both hospital staffs and patients. Healthcare professionals should actively take part in educating patients and visitors. One study showed that CDI can be reduced by educating patients about hand hygiene (24). Isolating patients upon the occurrence of symptoms, along with following the contact precautions and strict antimicrobial stewardship, will help reduce infections. Environmental cleaning with 1:10 dilution of bleach or sporicidal agents should be followed. Also, patients should be advised to avoid placing high-touch objects on the floor.

5.3. Limitations

It was difficult to identify the data collected for bed tracing. Although we were able to manually extract the data for all negative patients, we were unable to continue in a more dynamic form, such as creation of heat map for visual analysis. The room culturing was carried out in real time, some of the rooms were occupied and some were not, and some of the unoccupied rooms did not have beds. Hence, there was no uniformity in culturing. This study was conducted in a single center, therefore the data may not be generalizable.
5.4. Further research

Future research should focus on identifying a strategy to analyze bed tracing data in a more dynamic form. Molecular typing of isolates from CDI patients will help us identify clonality. Also, culturing of rooms before and after a patient stay or before and after room cleaning would help us identify the lapses and improvements required in environmental cleaning.
6. CONCLUSIONS

Bed tracing is a powerful tool to highlight highly positive rooms and could be used to detect the spread of CDI within the hospital. Creating heat maps would be of more value. Even though the cleaning protocol used in the hospital is constantly updated to match the infection control standards, there were still some rooms and wheelchairs that were positive for CD. This shows the need to enhance cleaning protocol followed in the hospital and to use sporicidal agents to clean the floors. Also, routine checks using cultures would be a good strategy to ensure adequate environmental disinfection. It is possible to prevent CDI in a healthcare setting by following an effective infection control and antimicrobial stewardship programs.
## APPENDIX: Data Collection Tables

Table 6: Excel table used for identifying the baseline characteristics and for bed tracing

<table>
<thead>
<tr>
<th>Subject id</th>
<th>Age</th>
<th>Birth Date</th>
<th>MRN</th>
<th>Sex</th>
<th>Race</th>
<th>Death Date</th>
<th>Possible HAI</th>
<th>Possible CAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CI</th>
<th>CI Conditions</th>
<th>Readmission (0/1)</th>
<th>No of times readmitted (0/1/2)</th>
<th>Antibiotics (0/1)</th>
<th>List of antibiotics</th>
<th>PPI (0/1)</th>
<th>Date of +ve CDI test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>History of CDI, 2014 and or 2015 (0/1)</th>
<th>Dates, 2016 Admit date – Discharge date</th>
<th>Length of stay (from previous column)</th>
<th>Bed tracing Room number – Dates in the room</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Table 7: Table used to classify the patients in top-13 rooms

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>CDI status during stay</th>
<th>Duration : In and Out</th>
<th>Duration between each +ve stay</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD8241</td>
<td>Positive</td>
<td>2/13/2016 2/25/2016</td>
<td>0</td>
</tr>
<tr>
<td>PD2636</td>
<td>Positive</td>
<td>2/25/2016 2/27/2016</td>
<td>125</td>
</tr>
<tr>
<td>WM5546</td>
<td>Positive</td>
<td>7/1/2016 7/4/2016</td>
<td>10</td>
</tr>
<tr>
<td>MK2066</td>
<td>Negative</td>
<td>7/14/2016 7/20/2016</td>
<td>16</td>
</tr>
<tr>
<td>HG8103</td>
<td>Diff stay</td>
<td>8/5/2016 8/20/2016</td>
<td>36</td>
</tr>
<tr>
<td>MC1944</td>
<td>Negative</td>
<td>9/25/2016 9/26/2016</td>
<td>18</td>
</tr>
<tr>
<td>CJ2433</td>
<td>Negative</td>
<td>10/14/2016 10/16/2016</td>
<td>1</td>
</tr>
<tr>
<td>DJ7701</td>
<td>Negative</td>
<td>10/17/2016 10/18/2016</td>
<td>56</td>
</tr>
<tr>
<td>KT7947</td>
<td>Diff stay</td>
<td>12/13/2016 12/14/2016</td>
<td>78</td>
</tr>
<tr>
<td>BM0355</td>
<td>Diff stay</td>
<td>3/2/2017 3/3/2017</td>
<td></td>
</tr>
</tbody>
</table>

Room 6: U3E1 3010 05

Average duration between each CDI patient stay: 37.8

Percentage of patients negative during the room stay and later became CD positive: 40%
BIBLIOGRAPHY


13. Muto, C. A., Pokrywka, M., Shutt, K., Mendelsohn, A. B., Nouri, K., Posey, K., ... Harrison, L. H. (2005). A Large Outbreak of Clostridium difficile–Associated Disease with an Unexpected Proportion of Deaths and Colectomies at a Teaching Hospital Following Increased Fluoroquinolone Use. *Infection Control & Hospital Epidemiology, 26*(03), 273-280. doi:10.1086/502539


