**THE PATHOGENESIS, EPIDEMIOLOGY, AND PUBLIC HEALTH SIGNIFICANCE OF CLOSTRIDIUM DIFFICILE**

by

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**ABSTRACT**

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Public Health Relevance: This literature review seeks to discuss the overall cost burden, impact, epidemiology, pathogenesis, preventative measures to assist in understanding *Clostridium difficile* as a pathogen and implement further effective measures of prevention and policy.

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*Clostridium difficile* is well-known as a hospital-acquired infection (HAI). By means of its toxins TcdA and TcdB, *Clostridium difficile* causes debilitating illness in hospitals. *Clostridium difficile* is known to be resistant to a number of antimicrobial agents, which further complicates treatment. Recurrent *Clostridium difficile* infection (CDI) is oftentimes treated with fecal transplants, which has been shown to be highly effective at curing recurrent CDI. Treatment, however, is known to incur high costs overall every year. Because of this, prevention methods are essential to controlling the transmission of disease and associated costs. Prevention should revolve around environmental sanitation through use of UV light treatment and sporicidal cleaners. Prevention methods should also target behaviors (i.e. hand-hygiene, antimicrobial prescription frequency) through education of healthcare workers and patients alike.

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# 1.0 Introduction

This essay was completed in fulfillment of the requirements for conferral of the Master of Public Health in Infectious Diseases and Microbiology (specialization in Pathogenesis, Eradication, and Laboratory) and Infection Prevention and Control practicum requirements as outlined in the student’s “Field Practicum Learning Agreement.” Student successfully completed all security and volunteer clearances (i.e. Blood-Borne Pathogens Training, EMTALA, HIPAA Policy Clearances, UPMC Confidentiality and Assignment Agreement). Objectives of the field practicum include:

1. Understanding the pathogen *Clostridium difficile* and analyzing the public health impact it has on healthcare costs and patient outcomes.
2. Describing how novel versus standard cleaning measures impact the pathogenesis, transmission, and presence of *Clostridium difficile* in healthcare locations.
3. Analyzing the role of patient and provider hand hygiene in hospital based transmission of the pathogen.

Objectives were fulfilled in the following ways:

1. Analysis of scientific literature from reputable sources – this helped facilitate understanding in how the pathogen establishes infection within the host, the basics of transmission, available treatments, and effects on healthcare costs. Metrics to be analyzed include antibiotic type, patient length of stay, daily defined doses, and mortality.

Literature review of different cleaners (oxicide, chlorox, and Tru-D UVC) - student was able to understand how different sanitation methods affect transmission and incidence

**2.0 SYMPTOMOLOGY AND DIAGNOSIS**

*Clostridium difficile* is a Gram-positive bacterium that has a major public health impact as a nosocomial infection. It establishes infection through consumption of bactericidal-resistant spores from the environment or contact with healthcare workers and is diagnosed using enzyme immunoassay (EIA) for presence of toxins A and B and stool cultures.1,2 Other methods of diagnosis include toxin testing via detection of glutamate dehydrogenase and PCR.2 Symptoms of *Clostridium difficile* infection include watery diarrhea (at least three bowel movements per day for 2+ days), fever, appetite loss, nausea, and abdominal pain/tenderness.3 Other less common symptoms associated with CDI include cramping and peripheral leukocytosis.2 The definition for a case of CDI includes passage of 3 unformed stools in 24 or fewer consecutive hours and stools positive for *C. difficile* toxins or colonoscopy or histopathology findings establishing pseudomembranous colitis.2

* 1. **BEHAVIORAL PREVENTION**

Preventative measures are an essential part of controlling CDIs in hospital settings. Practice guidelines from 2010 establish a number of preventative measures. Healthcare workers and visitors are to wear gloves and gowns upon entering a CDI patient’s room.2 Appropriate hand hygiene (i.e. use of antimicrobial soap and water) is to be enforced.2 Contact precautions are to be made known to hospital staff and visitors and isolation should be enforced.2 In regards to environmental surfaces and disinfection, disposable medical instruments should be employed and chlorine-containing cleaning agents/sporicidal products should be utilized in areas of contact with CDI patients.2 In regards to physician behavior, antimicrobial prescription regiment frequencies and duration should be minimized.2 Antimicrobial stewardship programs should be implemented with special emphasis on restriction of cephalosporins and clindamycin2. The combination of behavioral awareness/education for visitors and physicians, enforcement of personal protective equipment regulations, and environmental sanitation with appropriate agents make these measures particularly effective in preventing CDI.

**4.0 ENVIRONMENTAL PREVENTION: UV LIGHT TREATMENT**

Environmental cleanup and sanitation are also effective preventative measures in reducing hospital-acquired *Clostridium difficile*. One method of hospital decontamination includes use of the Tru-D SmartUVC system, which uses UV light as a form of disinfection. One trial, which used a Formica sheet, was inoculated with approximately 104 – 105 *Clostridium difficile* pathogens.4 Within 15 minutes, 99.9% of vegetative bacteria were killed.4 99.8% of *C. difficile* spores were killed within 50 minutes.4 Other trials have found the Tru-D/UV treatment to reduce *C. difficile* cultures by 80%, a statistically significant reduction in spores on hospital surfaces.5 Initially, the Tru-D required long durations (approximately 44 minutes) to effectively disinfect patient rooms of *Clostridium difficile* spores, making this not very time-efficient.4 However, with the addition of nonstructured UV-reflective wall coating, a 2.91-log reduction of spores (2.78-log without the reflective coating) and reduction of the disinfection cycle time to 9 minutes and 24 seconds could be achieved.6 UV light treatment is an effective way to reduce *Clostridium difficile* spores on hospital surfaces.

**5.0 ENVIRONMENTAL PREVENTION: CLEANER SOLUTIONS**

Environmental disinfection methods also include the use of cleaner solutions. Many hospitals utilize Clorox Healthcare® Bleach Germicidal Wipes (composed of 0.55% of sodium hypochlorite) and Clorox Bleach (1:10 dilution) as these have been proven effective in removing and inactivating spores (meaning a greater than 3 log10 decrease).4 Kill times against *Clostridium difficile* spores for Clorox Healthcare Bleach Germicidal Wipes have been found to be approximately 3 minutes.7 Another environmental cleaner that is effective against *Clostridium difficile* is OxyCide, a peracetic acid/hydrogen peroxide-based disinfectant solution. Studies have shown significant reductions of *Clostridium difficile* incidence rates within hospitals as a result of OxyCide treatment.8

**6.0 EPIDEMIOLOGY**

*Clostridium difficile* is strongly associated as a hospital-infection, as rates among the hospitalized range from 25-55% while rates among the nonhospitalized are typically less than 5%.1 The rates of *Clostridium difficile* infection have increased more than two-fold in the period spanning from 2000 (3.82 infections per 1000 discharges) to 2008 (8.75 infections per 1000 discharges) with the elderly (those older than 65) being at highest risk.1 It is generally understood that nearly 20-27% of all CDI cases are community-acquired and incidence ranges from 20-30 cases per 100,000 patients.1 In regards to mortality, the age-adjusted death rate experienced a 15% increase in deaths from 2007 (2 deaths per 100,000 patients) to 2008 (2.3 deaths per 100,000 patients).1 Two special populations of discussion in regards to CDI are children and peripartum women.1 Children 1-4 years of age are at greatest risk of infection.1 Overall, an increase in incidence has been observed in pediatric populations from 0.724 cases per 1,000 hospitalizations (1997) to 1.28 cases per 1,000 hospitalizations (2006).1 Risk factors for CDI in pediatric populations include comorbidities such as cancer, solid organ transplant, and gastrostomy.1 In peripartum women, incidence rates increased from 0.04 cases (2004) to 0.07 (2006) cases per 1000 discharges.1 Cesarean section delivery seems to be a risk factor for peripartum women.1

**7.0 RISK FACTORS**

Generally speaking, there are quite a few factors associated with elevated risk of *Clostridium difficile* carriage and diarrhea.9 Duration of hospital stay was found to be significantly associated with increased risk of infection.9 It was found that a hospital stay of 15-114 days resulted in 5.09 times greater risk of contracting *C. difficile* than a hospital stay of 2-4 days.9 Univariate analysis determined that host factors such as laxative abuse history, previous hospital-acquired infections, and being immunocompromised increased risk of carriage (aRR of 4.24, 1.75, and 1.52, respectively) and diarrhea (aRR of 3.92, 3.44, 1.85, respectively).9 Stool softeners were found to increase carriage by 2.30 times and gastrointestinal stimulants increased risk of diarrhea by 5.18 times.9 Procedures such as nasogastric tubes, enemas, nongastrointestinal surgeries, and endoscopies all increased risk of carriage (aRR of 2.36, 2.27, 2.11, and 1.84, respectively) and diarrhea (aRR of 3.6, 2.36, 2.34, 2.71 respectively).9 Additionally, Vancomycin antibiotic regiments were found to increase risk of diarrhea by 3.08 times, with TMP/SMX increasing risk of diarrhea by 2.67 times.9

**8.0 HEALTHCARE COST BURDEN**

*Clostridium difficile* infections contribute significantly to healthcare costs. Based on a systematic review, it has been estimated that CDI contributes to between $750 million and $3.2 billion to healthcare costs worldwide.10 In the United States, it is estimated to cost between $433 to $797 million per year.10 Individual cases of primary CDI treatment were found to cost between $9,822 and $13,854.10 Recurring cases found costs to be approximately three times higher when compared to primary cases.10 These estimates make a strong case for emphasizing preventative measures in hospital settings.

**9.0 BACTERIOLOGY OF *CLOSTRIDIUM DIFFICILE***

The bacterium produces two primary toxins: TcdA and TcdB.11 These bacterial toxins inactivate the host G-proteins Rho, Rac, and Cdc42, all of which are involved in growth and division in the target cell.11 TcdB is TcdA dependent, which essentially means that without TcdA, TcdB will not cause disease.11 These proteins are encoded by two genes: *tcdA* and *tcdB*, both of which are found in the 19.6 kb pathogenicity locus of the *C. difficile* genome.11 The two toxins share a great deal of structural homology (74% to be exact) and this homology is shared specifically in the enzymatic and receptor binding domains of the two toxins, giving credence to the argument that the two demonstrate similar substrate specificity.11

Environmental interaction plays a big role in expression of the toxins. Limitations on biotin availability has been found to increase TcdA concentrations 35-fold and TcdB 64-fold.11 Additionally, the toxins may be upregulated during times of stress for the bacteria (i.e. when the host is taking an antibiotic regiment).11 It was found that antibiotics at sub inhibitory levels actually upregulate the toxin expression.11

The two toxins target Ras GTPases and inhibit their activity via glycosylation.11 TcdA’s mechanism involves binding to nonproteinaceous receptors with the disaccharide Galβ1-4GlcNac.11 After binding, the toxins enter the target cell via receptor-mediated endocytosis and are acidified in an endosome.11 Once acidified, TcdB causes actin cytoskeletal reorganization, which can oftentimes prompt cell death.11 Via the process of glucosylation, TcdB can inactivate RhoA, Rac, and Cdc42.11  Functioning on the same principle, TcdA modifies RhoA, and inactivates small GTPases, prompting expression of caspases 3 and 9 (both of which are pro-apoptotic molecules).11 All these modifications result in cell rounding, blebbing, and apoptosis.11 Experimentally, it was found that TcdB induces adverse cellular effects at a faster rate than TcdA.11 Both toxins have been found to cause cytoskeletal reorganization and stress fiber localization at tight junctions, increasing permeability in the intestinal apical epithelium.11

**10.0 HOST IMMUNOLOGICAL INTERACTION**

Immunologically, the host is found to mount cytokine responses. TcdA is primarily responsible for the inflammatory events through induction chemokines in human intestinal epithelial cells and activation of mitogen-activated protein kinases, leading to production of IL-8.11 A combination of increased epithelial permeability, chemokine and cytokine production, reactive oxygen species (ROS) release, and mast cell production all contribute to damage in the intestinal mucosa due to pseudomembranous colitis.11 This damage is accompanied by colonic inflammation, death of epithelial lining, and neutrophilic activity.12 Additionally, TcdA has been found to activate enteric neuronal cell lines.12 Neuropeptides such as SP and CGRP have demonstrated to facilitate fluid secretion and membrane permeability, as evidenced by neuropeptide inhibitor experiments.12 Increased fluid secretion and membrane permeability manifest into the diarrhea characteristic of *C. difficile* infection. TcdA elicits secretion of leukotrienes, PGE2, and IL-8.12 These molecules play a significant role in signaling cascades to activate mast cell activity.12 These mast cells then activate the neutrophilic response and cause aggregation of the neutrophils in the epithelium space.12 To protect itself, the host elicits a nitric oxide release, which helps to decrease sugar permeability and fluid secretion.12 In essence, the nitric oxide response protects the mucosal lining, as it downgrades the rate of mast cell degranulation and neutrophilic activity associated with TcdA.12

**11.0 ANTIBIOTIC RESISTANCE AND TREATMENT**

*Clostridium difficile* treatment is complex and involves numerous factors. *Clostridium difficile*  is known to have developed antibiotic resistance, oftentimes making treatment difficult and inefficient. Strain 630, is one antibiotic resistant strain.13 The genome is characterized by a circular chromosome of 4,290,252 base pairs and a plasmid (pCD630) of 7,881 base pairs.13 Coding sequences unique to *Clostridium difficile* are known to have accessory functions and many mobile elements.13 One key characteristic that mediates antibiotic resistance is the presence of conjugative transposons.13 These transposons do have genes that encode antibiotic resistance. One such gene contained in these transposons is Tn5397, which plays a role in tetracycline resistance.13 Other transposons are CTn2, CTn4, and CTn5, all of which are related to Tn1549 (a transposon related to vancomycin resistance).13 Other coding sequences involved in antimicrobial resistance include CD0456 (which confers daunorubicin resistance), CD0643-646 (which confers bacitracin resistance), CD3215 (nogalmycin resistance), and CD0458 (beta-lactam resistance).13 In summary, the genome of antimicrobial resistant strains of *Clostridium difficile*  are composed of highly mobile and integrated genetic elements.

One antibiotic to use in treatment is fidaxomicin.14 Fidaxomicin combats bacteria by binding to the RNA polymerase and inhibiting the subunit, thus preventing subunit recognition by the promoter.14 Dosages required for MIC50 (minimum inhibitory concentration for 50% of organisms) and MIC90 (minimum inhibitory concentration for 90% of organisms) was found to be lower for fidaxomicin when compared to metronidazole and vancomycin (two other antibiotics used in the treatment of CDI).14 In contrast to other antibiotics (specifically vancomycin), fidaxomicin does not significantly alter the normal intestinal microbial flora.14 This is crucial, as *Clostridium difficile* thrives only when there is little competition in the gut. As of now, no fidaxomicin resistance has been observed in microbial populations, making this an effective candidate against mild-to-moderately severe CDI.14 Prior to 2012, metronidazole was used predominantly for treatment of first episodes of mild-to-moderate CDI at a dose of 500 mg 3 times per day for 10-14 days, while Vancomycin is predominantly used for the first severe CDI episode at a dose of 125 mg orally 4 times per day for 10-14 days.2 Recommendations have been adjusted accordingly to include fidaxomicin.

Another form of treatment of CDI what is popularly known as “rePOOPulating” the gut.15 While the antibiotics aforementioned are involved in single episodes or subsequent episodes, this therapy is used only for recurrent CDI, meaning a subsequent infection after complete resolution while on antimicrobial therapy.15 This therapy is formally known as fecal bacteriotherapy or stool transplants.15 The rationale behind this treatment is to re-establish normal intestinal microbial flora.15 Stool transplants involve the use of culturing the healthy microbial flora of a healthy individual.15 The more diverse the flora, the better the outcome.15 In an initial trial, 62 isolates were recovered on media.15 Additionally, to prevent complications, *C. difficile* was isolated from the stool samples via selective media.15After colonoscopy the evening before the procedure, the stool solution was administered into the colon and sent home, being instructed to avoid probiotic-containing food and eat a high fiber diet.15 The results of the trial found that normal bowel pattern returned in 2-3 days and patients treated remained symptom-free at 6 months, giving strength that fecal transplants can effectively cure recurrent CDI.15 Small, uncontrolled studies have found success rates of fecal transplants to be approximately 89%.16

**12.0 HAND-HYGIENE INTERVENTION**

Hand-hygiene is an important component involved in the transmission of *Clostridium difficile* in hospital settings. The World Health Organization describes 5 Moments of Hand Hygiene17:

1. Before patient contact
2. Before an aseptic procedure
3. After body fluid exposure risk
4. After Patient Contact
5. After Contact with Patient Surroundings

The rationale behind moment one is to protect patients against harmful pathogens on a healthcare worker’s hands.17 Moment two is in place to protect patients against pathogens on both the healthcare worker’s skin and the patient’s skin.17 Moments three to five protect the healthcare workers and the hospital environment.17

 A four-year intervention known as the “Cleanyourhands” campaign found significant reductions in *Clostridium difficile* hospital incidence with increased hand-hygiene compliance among healthcare workers.18 The intervention focused on education (posters), providing feedback on compliance in the form of audits, and alcohol hand rubs at patient bedsides.18 Evaluation of the intervention used a prospective, ecological, time series design.18 Volume of remaining alcohol and liquid soap at the end of the month was used to benchmark usage and compliance of hand hygiene policies.18 The results of the intervention found soap and alcohol hand sanitizer compliance to increase considerably (from 17.4 mL to 33.8 mL per patient bed day for alcohol and from 21.8 mL to 59.8 mL per patient bed day for soap).18 Increases in healthcare worker hand-hygiene compliance coincided with decreased rates of CDI (infection rates fell from 16.75 to 9.49 cases per 10,000 bed days).18 It is important, therefore, to emphasize hand-hygiene procedures on both the patient and healthcare worker populations.

**13.0 TWELVE MONTH SURVEILLANCE RESULTS**

Average incidence rate for *C. difficile*  in the fiscal year was determined to be approximately 8.68 per 10,000 patient days (pt. days). Average patient hand-hygiene for the 2014-2015 fiscal year was determined to be 85.90%. Data was obtained from Infection Prevention and Control staff at UPMC Shadyside.

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**Figure 1. FY 15 Hospital C. diff Incidence per 10,000 pt. days**

Figure 1: Beginning in January 2015, the CDC changed the definition of a case. Patients no longer need to present with symptoms but rather must test positive in a lab culture. The rate was determined using the formula Rate Calculation: [Total *C. difficile Incidence*/Patient Days] x10,000 patient days.

**14.0 DISCUSSION AND FUTURE RECOMMENDATIONS**

Figure 1 suggests an association between hand-hygiene compliance and *C. difficile* incidence. Between January 2015 and February 2015: fairly steep increase in compliance coupled with decrease in incidence. This trend holds true between the months of August 2014 and September 2014, November 2014 and December 2014, and May 2015 and June 2015. While no statistical analysis could be conducted, there are possible associations with hand-hygiene education and *Clostridium difficile* incidence at Shadyside hospital.

 Literature has established the benefits of sporicidal and bleach methods in lowering *Clostridium difficile* incidence in hospitals. It is recommended, therefore, to include bleach wipes for patients to use to wipe down hospital surfaces. The Tru-D system should be utilized at least twice a week in CDI patient rooms (whenever the patient is absent from the room). This will help keep spore concentrations to a minimum and prevent healthcare workers from acquiring the spores and potentially transmitting it to other patients. In regards to the importance of hand-hygiene, patient and staff education is crucial. Distributing materials in regards to hospital-acquired infections is one way to inform the patient population of the risks associated with hospital-acquired infections. Daily reminders from Infection Control staff, nurses, physicians, and food workers regarding the importance of hand hygiene are also helpful in educating vulnerable populations. Upon admittance to inpatient floors, patients should receive information on hand-hygiene and HAI’s that come with lack of hand-hygiene. Meals should also include friendly reminders to utilize soap and water or hand sanitizer wipes before food consumption. To maintain high compliance among staff, units should be audited on a random rotation system, in which it is audited at least twice every month to assure quality. Rather than observing 15 moments of care, at least 25 observations of moments of care should be conducted to ensure the unit’s staff is compliant.

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