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Clostridioides difficile Infection - Understatement of General Environmental Risk and Overstatement of Healthcare Environment Risk

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Abstract

Clostridioides difficile infection (CDI) causes a significant amount of morbidity and mortality in the US and globally. Historically it has been believed that a most healthcare onset CDI (HO-CDI) is a result of environmental exposure to C. difficile spores while in a healthcare facility, resulting in hospital onset C. difficile infection. Emerging evidence suggests that this view overstates the risk of patient-to-patient transmission, whether transmitted directly or via contaminated surfaces or hands. This paper discusses the current state of the evidence of C. difficile transmission through potential exposure within a healthcare environment, the risk of colonization through other potential environmental exposures outside of healthcare facilities, the prevalence of environmental shedding from colonized and infected patients and whether colonized or infected patients are likely to transmit C. difficile to other patients. Specific to the potential role of the healthcare environment, the paper reviews the limited impact improved cleaning and disinfection programs have been shown to have on CDI rates.

Introduction

Clostridioides difficile (formerly *Clostridium difficile*) or *C. difficile*, is a Gram-positive, spore forming, anaerobic rod-shaped bacterium (bacillus) that causes a significant amount of morbidity and mortality both in the US and around the world (Leesa, 2015) (Knight, 2015). Conventional thinking on the epidemiology of *C. difficile* is that a significant portion of transmission occurs in hospitals, resulting in hospital onset *C. difficile* infection (HO-CDI). This is considered a Healthcare Associated Infection (HAI) and traditionally it was assumed that this transmission primarily occurs horizontally between symptomatic patients. This thinking is based in large part on the supporting evidence from historical studies using polymerase chain reaction (PCR) ribotyping of clinical samples, which has been used to show that patient clinical samples often share the same ribotype (Knight, 2015). Also, some studies show that proximity to infected patients increases the risk of subsequent infection in other patients (Shaughnessy, 2011).

This epidemiological view is reflected in current *C. difficile* guidelines in the US and Europe (Dubberke, 2014) (McDonald, 2018) (Tschudin-Sutter, 2018) and is generally accepted in the scientific literature. However, emerging evidence suggests that this thinking overstates the true risk of patient to patient transmission, whether direct or through an intermediate host, such as contaminated

healthcare worker hands on shared medical equipment (Eyre, 2013) (Brown, 2015) (Walker, 2012). Newer evidence is demonstrating the limits in this prior understanding of person-to-person transmission. Undoubtedly some amount of HO-CDI is facilitated by patient-to-patient transmission, either directly or indirectly through contaminated hands or surfaces, but this is likely to be much less significant in causing CDI (i.e. 10-20% of HO-CDI) than other sources for acquisition of *C. difficile* (Eyre, 2013) (Brown, 2015).

This paper discusses the current state of the evidence of *C. difficile* transmission through potential exposure within a healthcare environment and the risk of colonization through other potential environmental exposures. The paper also discusses the prevalence of environmental shedding from colonized and infected patients, whether healthcare workers are likely to transmit *C. difficile* to patients, and whether colonized or infected patients are likely to transmit *C. difficile* to other patients. Specifically to investigate the role of the healthcare environment, the paper reviews the impact improved cleaning and disinfection programs have on CDI rates and how better molecular epidemiological methods, such as whole genome sequencing (WGS), are providing new tools to better understand the pathways through which patient to patient transmission occurs in a healthcare facility.

Healthcare Transmission of *C. difficile*

For HO-CDI to be an HAI, a patient would have to be admitted without symptoms and develop symptoms of clinical infection more than 3 days after admission (CDC, 2021). For this infection to actually be healthcare transmitted, the patient would either have to be exposed to *C. difficile* after admission and move from colonized to infected more than 3 days after admission or have been exposed to *C. difficile* in a prior healthcare contact and become colonized, with the current healthcare contact resulting in moving from colonization to infection. Because screening for colonization is not generally done on admission, it is not currently possible to determine the portion of patients already colonized on admission.

Screening on admission to a hospital could include medical history of hospitalization in the past 6 months, prior antibiotic use, and other medications that might increase the risk of developing CDI, which could be used to test high risk patients for colonization. Some facilities are performing routine screening for *C. difficile* colonization on inpatient admission to identify higher risk patients. Collison (2021) detected colonization in 4.2% of patients during a three-year study in an urban medical center.

While not required currently, for HO-CDI to be an HAI there should be genetic evidence that the same strain of *C. difficile* was present in another patient and that there was some opportunity for transmission to occur. Ongoing surveillance can identify if transmission in the facility is likely and patient assignment records can determine whether transmission via a HCW is possible. Genetic testing can provide confirmatory or contrary evidence in the investigation.

A study by Walker (2012) found that 66% (465 of 705) HAI cases were unlinked genetically and 23% (165 of 705) had a potential genetically linked patient. However, after adjustments for chance meetings between the patients and an assessment of likely transmission events,

Walker estimated only 16% of cases were linked by probable transmission events. While still a significant portion of cases to be addressed through infection prevention activities, it is clearly a smaller portion of the total CDI cases. Walker further commented that there was no evidence of significant onward transmission from contaminated wards after patient discharge, that the use of single patient rooms likely lowers the risk of patient-to-patient transmission, and that if multiple transmissions occurred over a short period of time, they were confident their study model would have detected this transmission, which it did not.

Prior studies on *C. difficile* have shown that a person is likely shedding spores up to one week prior to diagnosis, during treatment, and up to 8 weeks after diagnosis (Eyre, 2013). Sethi (2012) showed that while treatment for CDI reduced the percentage of stool, skin, and environmental samples that tested positive for C. difficile, the percent of samples positive increased for up to 4 weeks after treatment, indicating prolonged shedding. This suggests the window of transmission is potentially quite broad and that prolonged persistence on environmental surfaces may be at least partially due to ongoing shedding, rather than poor cleaning practices in healthcare facilities. In the study by Eyre, they assumed that the window from colonization to infection could be up to 12 weeks, with some portion of recovered patients still testing positive for *C. difficile* in their stool up to 3 months after treatment (Eyre 2013). A study by Walker (2012) used a 26-week window to allow for a significant amount of time from initial colonization to infection.

One study used multilocus sequence testing (MLST) to perform genetic analysis of patient *C. difficile* samples. The study could only associate 25% of HAI CDI to a previously identified CDI patient (Crobach, 2018). A much larger follow-up study by Eyre (2013) conducted over 3.6 years and using WGS found that only 35% of HO-CDI cases were genetically associated with previous cases. Of 957 cases of HO-CDI, 65% (624 of 957) were genetically distinct and thus not related to healthcare exposure (Eyre, 2013). Of the 333 patients where there was a genetic link to a previous infection, 38% of the patients (126 of 333 genetically related cases or 13% of total cases) had potential ward contact with the prior patient, 2% (5 of 333) of these cases were linked only by possible environmental contamination after discharge or recovery of the prior patient, 9% (29 of 333) were not on the same ward, but were in the same hospital and 6% (21 of 333) had ward contamination and hospital contact (Eyre, 2013). Of the remaining 46% (152 of 333) patients, no hospital based contact could be determined (Eyre, 2013). This study suggests that exposure through the hospital is more likely to be an important, but minor factor in overall CDI risk and that CDI risk is not primarily transmitted from symptomatic patients, but may be acquired from asymptomatic people or some other environmental reservoir, most likely outside the hospital setting (Eyre, 2013).

Some studies show an association between exposure to colonized patients and developing CDI. Blixt (2017) reported on a study from Denmark where all patients were screened for *C. difficile* on admission and they found that the risk of developing CDI was higher for colonized patients than for uncolonized patients (OR=4.64). CDI was detected in 2.6% (n=630) of patients without exposure to colonized roommates or other

patients on the same ward with CDI and 4.6% (n=630) when exposed to colonized roommates or other patients on the same ward with CDI (OR=1.79) (Blixt, 2017). Of the 195 colonized patients, 39 different strains were detected using MLST and 5 of 25 colonized patients admitted more than once had 2 different strains (Blixt, 2017). Crobach (2018) discussed two smaller studies which found in one study 30% of new cases could be linked to previously identified cases and in the other study 29% of new cases linked to colonized (asymptomatic) patients. These studies suggest that while exposure to *C. difficile* spores while in a hospital are an important risk factor, it does not account for the majority of transmission risk. A study by Murray (2017) found that for patients with CDI, exposure to the computed tomography scanner in the emergency department was associated with an increased risk (odds ratio = 2.7) of the patient subsequently developing CDI, suggesting a stronger association with environmental exposure. Pai (2020) reported on a study investigating spatiotemporal clustering of CDI cases and found that clustering of cases did occur in some units, but did not include WGS to test whether the cases where genetically related. This suggests environmental influences in some portion of CDI cases, but could not determine which factors most strongly influenced rates or what portion of cases were environmentally linked.

A review article by Durovic (2018) of studies of transmission of *C. difficile* in a healthcare facility identified the main sources of *C. difficile* acquisition as contact with symptomatic carriers (53.3%) and contact with the contaminated hospital environment (40.0%), but the reports offer conflicting results, and Durovic identified different study designs, the methodologies used to assess transmission, differences in infection prevention policies, differences in antibiotic prescription practices, and differences in facility infrastructure as likely drivers in the differences in results. Durovic (2018) reviewed a series of studies that investigated the causes of CDI and found asymptomatic carriers were the source of the infection in 9.8% of cases in one study and in another study 10.1% of CDI cases were linked to transmission within the hospital. In a large epidemiological study reviewed by Durovic (2018), 82% of CDI cases has a prior outpatient healthcare exposure and in another similar study, 94% of patients with CA-CDI had outpatient exposure to a healthcare facility in the prior 12 weeks. This is a common theme in the literature, finding that CDI patients, whether classified as CO-CDI or HO-CDI, have had prior exposure to healthcare facilities. However, a lack of genetic evidence limits the ability to demonstrate a connection between the two events given the significant opportunities to become colonized with *C. difficile* outside of healthcare exposure.

Domestic and food animals as *C. difficile* host

When investigating the risk of person-to-person transmission in healthcare, we should consider all potential routes of exposure to *C. difficile*, including those that occur outside of a healthcare facility. Two unrelated people may become exposed to the same toxigenic strain of *C. difficile* from the same 'geographic' source, be admitted to a hospital for unrelated reasons, and go on to develop CDI while being treated in the same hospital. The temporal relationship of the infections may indicate that patient A transmitted the pathogen to patient B. However both patients may have been exposed to the same non-

healthcare source of the pathogen prior to hospitalization. For *C. difficile*, it appears increasingly likely that this type of exposure may be under-considered, and that exposure increasingly appears to occur outside a healthcare facility.

There is a growing body of evidence that people are likely routinely exposed to *C. difficile*, including toxin producing strains, in their daily lives. While patients may become colonized with *C. difficile* during hospitalization or through exposure to healthcare facilities, this is not the only way people become exposed to toxigenic *C. difficile*. CA-CDI has been increasing globally and, in some areas, accounts for up to 25% of all CDI cases, commonly lacking classic risk factors such as healthcare or antibiotic exposure (Knight, 2015).

C.difficile has been found in animal sources including cats, dogs, pigs, cows, sheep, goats, chickens, horses, rabbits, wild birds, raccoon, zebras, kangaroos, elephants, monkeys, and chimpanzees (Knight, 2015). Transmission by farm animals to humans can occur as well. Knetsch (2014) showed pig colonization with the same strain in 42% of human farm workers in a study covering 2002-2011 in the Netherlands.

Pets have also been identified with *C. difficile* colonization. Crobach (2018) reviewed a series of studies that found rates of asymptomatic colonization of pets with *C. difficile* ranged from 11-40%. In a study by Loo (2016), there was evidence of pet to owner transmission of *C. difficile* in 20% (3 of 15) domestic pet contacts and none of the pets had active diarrhea. Janezic (2018) investigated dog paws, shoes, and slippers in houses with dogs and found *C. difficile* present in 34% of samples with 43% of shoes, 28% of slippers, and 24% of dog paws contaminated with *C. difficile*. PCR ribotyping identified 13 different ribotypes with 5 being toxigenic (Janezic, 2018) (Durovic, 2018). Exposure from pets may affect *C. difficile* colonization risk. Lefebvre (2006) found 58% (58 of 102) of pet dogs that were allowed to visit a hospitalized patient, were colonized with *C. difficile*, and 71% (41 of 58) had toxigenic strains.

In an evaluation of bovine, porcine, equine and canine samples for *C. difficile* infections through PCR ribotyping, Keel (2007) identified 209 different *C. difficile* isolates across 13 ribotypes. Ribotype 078 was predominant among 83% (119 of 144) and 94% (31 of 34) of porcine and bovine isolates respectively but was found in only 4% (1 of 23) of human isolates from patient samples collected from Louisiana and Colorado (Keel, 2007). Keel (2007) also found a strong correlation between canine, equine and human ribotypes as 22% (5 of 23) of samples collected from humans tested positive for the ribotype 020, which was found in 17% (2 of 12) and 5% (1 of 20) of canine and equine samples respectively (Keel, 2007). This suggest that domestic animals that are routinely in close proximity with humans may play a significant role in the occurrence of human colonization and CA-CDI and presumably have the potential to repeatedly recolonize people. Keel (2007) also reported that 42% (5 of 12) of *C. difficile* isolates from canines had ribotypes identical to those isolated from 13% (3 of 23) of human isolates, suggesting a relationship between people and dogs for colonization. Not all studies find animals as a significant source of *C. difficile* exposure. In a study of likely sources of CA-CDI, Weese (2010) concluded that dogs were not a significant source of CA-CDI as *C. difficile* was isolated in only 10%

(14 of 139) of the dogs sampled. The ribotype 078 previously reported by Keel in their 2007 US study was later reported by Bauer (2011) as the most prevalent *C. difficile* strain detected in 2009 among European residents.

Since the gastrointestinal tract of domestic animals, such as cats and dogs, may harbour *C. difficile*, the risk of shedding *C. difficile* onto environmental surfaces should be considered (O'Neill, 1993). O'Neill (1993) found considerable similarities between *C. difficile* isolates from pets and environmental surfaces in veterinary hospitals and approximately 50% of isolates were toxigenic (O'Neill, 1993), suggesting that environmental surfaces in the vicinity of pets may serve as secondary host of toxigenic *C. difficile* isolates and may present a risk to humans. In an extensive review of *C. difficile* in food and animals, Rodriguez (2016) reported that asymptomatic domestic and food animals routinely test positive for toxigenic strains of *C. difficile*, suggesting that *C. difficile* should be considered as a zoonotic pathogen. The European Food Safety Authority (EFSA) (2018) describes a zoonotic disease as an infection or disease that may be transmitted from animals to humans through direct or indirect contact (EFSA, 2018**).**

Investigating likely transmission pathways for toxigenic *C. difficile* strains for domestic animals, pet food has also been analysed for *C. difficile*. Freeman (2013) evaluated certain pet treats for bacterial contamination and found that 4% (1 of 26) were contaminated with *C. difficile,* 4% (1 of 26) with methicillin-resistant *Staphylococcus aureus,* and 4% (1 of 26) with tetracycline-resistant *Escherichia coli*. A study by Weese (2005) found that 4% (1 of 25) of turkey-based dog meals sampled were contaminated with *C. difficile*. These studies continue to highlight the ubiquitous nature of toxigenic *C. difficile* isolates and the need for rigorous control measures to be enforced to mitigate *C. difficile* contamination of products that are rapidly becoming integral parts of every household.

Food and the Environment as potential vehicles for *C. difficile* transmission

In addition to potential animal exposures to *C. difficile*, it is also possible to become exposed to *C. difficile* through humans eating various foods, although current evidence of food to person transmission is limited and does not include genetic epidemiology, which would provide strong evidence of transmission. Because it is understudied, there is little evidence formally linking exposure to *C. difficile* for humans though eating food can result in *C. difficile* colonization. Studies of food, especially meat products, found certain retail meats (ground beef, ready to eat beef, ground pork, ground turkey, and pork sausage) contaminated with *C. difficile* in 20 to 63% of samples (Crobach, 2018). A study by Weese (2009) found samples of ground beef and pork positive for *C. difficile* in 12% of samples and that the bacterial load was 20 to 240 spores per gram of meat. Tkalec (2019) found 18.2% samples of vegetables contaminated with *C. difficile* with 28.0% of potatoes, 9.4% of leaf vegetables, and 6.7% of ginger roots contaminated, indicating foodstuffs other than meat have also been found to be contaminated with *C. difficile*. However, these studies did not confirm the presence of toxigenic strains.

Slaughterhouses and the hands of employees in food processing facilities have been implicated in the incidence of *C. difficile* on

food products. In an evaluation of carcasses at slaughterhouses, Rodriguez (2013) found that cattle carcass contamination rarely occurred at the slaughterhouse with 7.9% (8 of 101) of samples that tested positive for *C. difficile,* only one sample had both intestinal and carcass contamination. Environmental surfaces and equipment used at a slaughterhouse and slaughterhouse employees are thus another potential source of *C. difficile* contamination of carcasses at slaughterhouses, but this also requires further study.

A study from Zimbabwe by Berger (2020) investigated the presence of *C. difficile* in chicken feces and soil (80 samples) compared to patients treated for *C. difficile* within healthcare settings. Berger (2020) found, chicken and soil samples were positive for toxigenic strains 87% and 95% respectively, while the patient samples were positive 65% of the time but did not confirm through MLST or WGS the genetic relatedness of the strains.

The association between food and *C. difficile* contamination and subsequent development of colonization or infection is understudied in the literature. Since many foodstuffs have been shown to be *C. difficile* positive, including with toxigenic *C. difficile* strains, it seems likely some portion of colonization should be attributed to contaminated foods, but studies linking the two are rare. Since acute care and long-term care facilities rarely test their food supplies for *C. difficile*, it seems possible that they routinely feed hospitalized and nursing home residents' food contaminated with *C. difficile*, but this requires further study.

It is a matter of controversy whether finding *C. difficile* in environmental samples, such as water or soil samples reflects fomite contamination or a reservoir. Typically, a reservoir would provide sustenance for a bacteria and the ability to reproduce, while a fomite would reflect a contamination that would not inherently be a favorable environment for the bacteria. As *C. difficile* in anerobic (Crobach, 2018), it is unlikely to reproduce in oxygen rich environments. However, detection of *C. difficile* in environmental samples is quite common. A study of general environmental samples in Australia (soil, water, etc.) found 7.1% of samples were positive for *C. difficile* with water samples frequently contaminated in 36% of samples (Crobach, 2018). Another study in Australia (Moono, 2018) found 59% of lawn samples in public spaces contaminated with *C. difficile* and toxigenic ribotypes predominated the samples. A study in Canada found 39% of river sediment samples, which were connected to the discharge from a wastewater treatment plant, were positive for *C. difficile* (Crobach, 2018). An outbreak involving contaminated tap water was reported in Finland (Durovic, 2018).

It has also been postulated that healthcare workers may carry *C. difficile* spores into their homes. Otten (2010) proposed a transmission model for CA-CDI in which they discussed the potential role of family members in person-to-person transmission with 19% of healthcare worker uniforms positive for *C. difficile* after a hospital shift and these workers subsequently taking the contaminated uniforms home to be laundered. While this does not prove transmission causing infection, it is another viable route for a person to become exposed to *C. difficile* spores and subsequently become colonized and needs further investigation. Healthcare facilities should

consider environmental exposure in addition to healthcare exposure as resulting in human colonization. While *C. difficile* is not an established foodborne pathogen, there is increasing evidence that *C. difficile* finds a niche in diverse food types. However, because *C. difficile* is not generally considered a foodborne pathogen risk, fecal samples from victims of foodborne illnesses during a suspected foodborne outbreak are not commonly tested for *C. difficile.*

Environmental Shedding of *C difficile* is Common in Colonized and Infected Patients

People with *C. difficile* spores in their intestinal tract, whether colonized or infected, frequently shed spores into the environment and have positive skin tests for spores as well. The amount of shedding varies depending on whether a patient is asymptomatically colonized, infected, or receiving antibiotic therapy for CDI, but the investigation into patient specific characteristics that may increase shedding is limited. Factors which may play a role, such as level of continence, whether the patient self-toilets or uses briefs, and fecal waste management practices are typically not monitored in studies.

Patients with CDI shed *C. difficile* spores while suffering from diarrhea, but much less so after completion of treatment (Crobach, 2018). Sethi (2010) studied 52 patients with CDI. Prior to treatment, 100% of patient stools and 90% of patient skin and environmental surfaces tested positive for *C. difficile*. After treatment started, patient stool became *C. difficile* negative after a mean of 4.2 days, while 60% of skin samples (chest and/or abdomen) and 37% of environmental samples remained *C. difficile* positive, suggesting lower amounts of shedding still occurred even when the amount in the stool was below the limits of detection (Sethi, 2010). Post-treatment testing of patients from 1-4 weeks after completion of treatment found 56% of stool samples, 58% of skin samples, and 50% of environmental samples were positive for *C. difficile* indicating ongoing colonization (Sethi, 2010).

Persistent shedding after treatment for CDI was associated with additional antibiotic therapy, often for reasons unrelated to CDI (Sethi, 2010). Sethi (2010) also reported that the mean density of *C. difficile* spores for a patient with CDI decreased from 5-6 $\log_{10}$ CFU/g prior to treatment to ~2 $\log_{10}$ CFU/g at the end of treatment and then levels increased at 1-4 weeks after treatment to 3.0-3.5 $\log_{10}$ CFU/g, suggesting that the antibiotic therapy reduces the amount of *C. difficile* inside the patient for a period of time, but does not prevent a post-treatment patient from shedding spores into the environment and that post-treatment antibiotics (often taken for unrelated medical issues) may actually increase the rate of shedding of *C. difficile* spores. Crobach (2018) and Brown (2015) both discussed how colonized patients may play a significant role in *C. difficile* spore dissemination because the number of colonized people is typically about three times higher than the number of people with active CDI in healthcare facilities.

Infant colonization has been reported with rates varying from 4 to 71% (Crobach, 2018) with a recent study that pooled data calculating 35% of infants <1 year of age were colonized with *C. difficile* with colonization rates peaking at 6 to 12 months and then declining towards adulthood. A study by Adlerberth (2014) found that 71% of colonized infants had toxigenic strains. A

study by Robilotti (2020) found that multilocus sequence testing (MLST) of 224 pediatric patients with CDI (35% was initially classified as HO-CDI) showed 80% were from the same single sequence (ST) type, but testing using WGS and investigation for potential contact that could result in transmission did not confirm any credible transmission between patients.

A study by Riggs (2007) of male long-term care residents found that of the 73 men participating in the study, 7% (5 of 73) had active CDI, while 51% (35 of 73) were colonized and asymptomatic with toxigenic *C. difficile*. Thus colonized residents outnumbered residents with active CDI by a ratio of 7 to 1 (Riggs, 2007). The stool of the men with active, symptomatic CDI had higher levels of *C. difficile* than those of colonized men at 5.6 log CFU/g versus 3.6 log CFU/g (Riggs, 2007). In the 6 month follow up period, 46% (16 of 35) were admitted to a hospital and 20% (7 of 35) developed CDI (Riggs, 2007) with 2 of the 7 having had CDI previously. The study also tested for skin and environmental contamination and found that 78% of patients with active CDI had at least one positive skin culture (groin, chest, and/or abdomen) and 78% had at least one positive environmental culture (Riggs, 2007). For asymptomatic carriers, 61% had at least one positive skin culture and 59% had at least one positive environmental culture. For non-carriers 19% had at least one positive skin culture and 24% had at least one positive environmental culture (Riggs, 2007). Using pulsed field gel electrophoresis (PFGE), 87% (13 of 15) skin samples were the same strain of *C. difficile* as the patient's stool, while for environmental samples 58% (11 of 19) were identical to the patient's stool sample. Using sterile gloves, the authors found that contact with a contaminated skin site resulted in transfer of *C. difficile* in 57% (8 of 14) of patients (Riggs, 2007).

The presence of significant numbers of colonized patients may make facilities consider whether it is appropriate to screen and decolonized patients colonized at the time of admission. However, decolonization of asymptomatic patients colonized with *C. difficile* is not recommended by the Society for Healthcare Epidemiology of America nor by the Infectious Diseases Society of America (SHEA/IDSA). In a recent guideline, Dubberke (2014) noted that decolonization of patients is ineffective and may lead to a higher risk of CDI for the patient in the future. The current version of the SHEA/IDSA guideline (McDonald, 2018) did not include this recommendation, but also did not discuss decolonization of asymptomatic patients.

Patients with CDI often have explosive diarrhea and may self-toilet, use a portable commode, or wear briefs/diapers depending on their mobility and acuity. Flushing of toilets in a patient room has been linked with environmental contamination. A study by Wilson (2020) using settling plates showed plate positives increased from 12.5% to 26.4% after a CDI patient used the room toilet and flushed the toilet with 66% of the rooms tested positive for *C. difficile* on at least one sampling plate. The counts of bacteria were similar at distances of 0.15 m, 0.5 m, and 1.0 m from the toilet, indicating the *C. difficile* dissemination was over a broad area.

Healthcare Workers and Risk of Transmission to Patients

If transmission of *C. difficile* occurs within a healthcare facility, then presumably healthcare workers (HCW) can be involved in the transmission process and may be at risk themselves. While HCW can become contaminated with *C. difficile* while caring for CDI patients and some small portion of HCW becoming colonized with *C. difficile*, there is little direct evidence that the HCW colonization is a result of exposure to CDI patients since WGS is rarely done to establish the link.

Kato (2001) tested Japanese healthcare workers and found 4.2% colonized with *C. difficile* while Van Nood (2009) tested healthcare workers and found 0% of handprint agar plates positive, but 13% of fecal samples positive for *C. difficile*.

A study by Landelle (2014) reported 24% of HCW caring for patients with CDI had hand contamination with *C. difficile.* When gloves are not worn, hand contamination rates have been reported to be 8 to 59% for HCW caring for patients with CDI (Crobach, 2018).

Aguirre-Garcia (2020) studied HCW diagnosed with CDI and found that exposure to CDI patients was not a significant risk factor, while exposure to antibiotics (RR=4.5) and taking proton pump inhibitors (RR=2.0) were statistically significant. If direct transmission from person to person were a significant risk factor, the authors expected being exposed to the patient environment for an actively shedding CDI patient to increase the risk of infection for HCW, but this link was not established.

Prior Bed Occupancy Studies and Impact on CDI Risk

If transmission of *C. difficile* occurs within a healthcare facility, then presumably being exposed to the same bed space or same patient room as a CDI patient could have a significant impact on infection risk for the next patient or roommate. Several studies have investigated the impact of the prior bed occupant or roommate on the risk of the subsequent patient developing CDI.

A study by Freedberg (2016) found that for patients developing CDI the median duration of prior bed occupancy was 3.0 days, the median duration of the bed being empty was 10 hours, and the median time from bed admission to CDI was 6.4 days. In their final model, the hazard ratio (HR) for the patient developing CDI was 1.22 if the prior bed occupant received antibiotics but was not affected by the prior patient actually having CDI. This lack of relationship may reflect better cleaning on discharge when the patient is known to have CDI or it may reflect the significance of a patient having antibiotics and subsequently shedding a range of pathogens into the environment. How this could lead to CDI for the next patient is unclear, but the lack of connection to the prior patient having CDI is a significant finding. The hazard ratio for the patient developing CDI was 4.2 when the patient was given antibiotics, suggesting this is a much more significant risk factor, but the prior bed occupant receiving antibiotics had a small but measurable increase in risk for the next patient in the bed. Other significant risk factors included patient age >70 (HR=1.40) the patient receiving acid suppression medicine (HR=2.14), receiving immune-suppressants (HR=1.50), treatment in the ICU (HR=1.94), increased creatine level

(HR=1.07), decreased albumin level (HR=1.29) and contemporaneous CDI (HR=3.99), which is an estimate of *C. difficile* colonization pressure given HO-CDI on the same ward during a patient's at-risk period (Freeberg, 2016).

Echaiz (2014) also studied whether having a roommate with CDI increased the risk for the patient in the other bed. The study found that 7.5% of patients developed CDI when the roommate had CDI, but only 3.2% of patients developed CDI when the roommate did not have CDI (Echaiz, 2014). This risk did not achieve statistical significance, suggesting only a moderate impact on CDI risk for a patient when the roommate has CDI, that the study was underpowered to detect the difference in risk, or other confounders that were not controlled. This study is consistent with the view that only a portion of CDI cases are related to environmental exposure to *C. difficile* during hospitalization.

In an older study, Dubberke (2007) investigated whether having other patients on the same ward with CDI increased the risk of developing CDI for other patients on the ward. The relative risk (RR) for other patients was high (RR=3.9) when exposed to 2-8 other patients on the same ward with CDI and very high (RR=9.7) when exposed to 9+ patients with CDI on the same ward (Dubberke, 2007). This suggests a stronger relationship between patient exposure to environmental contamination or HCW contamination with *C. difficile* than was seen in several other studies discussed in this section, but did not examine the impact of antibiotic use, and further demonstrates the mixed results in the literature.

Probably the best-known study of the impact of prior room occupant on CDI rates is a study done in an ICU by Shaughnessy (2011) which found that patients had a risk of CDI of 4.6% but if the prior patient in the same bed had CDI, this risk increased to 11.0% (hazard ratio=2.35). This study was done in a single site and only involved a 20 bed ICU and did not use genetic techniques to confirm relatedness of subsequent infections which are limitations in interpreting this study. Typically a larger percentage of HAIs occur to patients in ICUs because the patients are generally more seriously ill and receiving antibiotics, which both increase the risk of HAIs. This study suggests this risk is especially significant for CDI. The authors (Shaughnessy, 2011) monitored the time from admission to development of CDI and found that it was 12.5 days. Since risk of CDI is generally associated with a longer length of stay, this is consistent with our understanding of CDI risk. Because antibiotic receipt is associated with CDI risk, it is also interesting that 83.6% of the patients in the ICU were on at least one antibiotic and 46.7% of patients in the ICU were on 3 or more antibiotics (Shaughnessy, 2011). This is also consistent with our understanding of factors that increase CDI risk. Shaughnessy (2011) also comments that they stopped monitoring for antibiotic exposure after patients left the ICU, but development of CDI within 30 days was still counted as an HAI in their study, suggesting an important limitation in understanding the full impact of antibiotic exposure. Why the risk measured in this study is so much higher than in other studies is unclear but limiting the study to the ICU with high antibiotic use may play an important role.

Infection Prevention and the Impact of Improved Cleaning/Disinfection

A number of studies have attempted to decrease CDI rates by improving infection prevention practices, including a focus on better environmental hygiene. The largest of these studies is a 16 hospital study by Ray (2017) in which the authors studied the impact on HAI rates for CDI through improved cleaning compliance (using a covert fluorescent marker) and using sporicidal bleach wipes for daily and discharge cleaning for CDI patients. Several of the hospitals (4 of 8 control hospitals and 2 of 7 intervention hospitals) used bleach wipes for all discharge cleaning presumably to ensure efficacy against *C. difficile* before the next patient stayed in the room (Ray, 2017). The cleaning compliance on targeted surfaces using the fluorescent marker for discharge cleaning improved from 63% to 82% and the cleaning compliance on targeted surfaces for daily cleaning in CDI rooms improved from 52% to 69% (Ray, 2017). Using environmental sampling on 4 high touch surfaces, the percentage of CDI rooms with at least one positive *C. difficile* samples significantly decreased from 13% to 3% and the percentage of non-CDI rooms with at least one positive *C. difficile* sample decreased non-significantly from 3% to 2%. Despite these substantial improvements in cleaning compliance on targeted surfaces and the use of a sporicidal disinfectant, there was no significant difference in CDI rates during the intervention or post-intervention period and there was also no correlation between the percentage of positive samples for *C. difficile* after cleaning of CDI or non-CDI patient rooms and the incidence of HO-CDI rates. While there are a number of possible explanations for the lack of improvement in HO-CDI rates, such as failing to address the risk associated with asymptomatic carriers and unidentified confounding, a potential explanation is that there is a weak relationship between environmental contamination with *C. difficile* and patient risk of developing HO-CDI.

In a similar Australian study involving 11 hospitals, Mitchell (2019) implemented an environmental cleaning bundle to reduce HAI rates for certain pathogens including *C. difficile*. The study found that while the frequency of cleaning touch points improved from 55% to 76% in bathrooms and from 64% to 86% in bedrooms, CDI rates increased from 2.34 to 2.52 per 10,000 patient days, which was not statistically significant (Mitchell, 2019). Because the study did not mandate the use of a specific chemistry for disinfection in CDI patient rooms, it is difficult to determine whether changing to a sporicidal product as Ray (2017) did would have had an impact, but facilities were allowed to continue to use the product mix they currently had in place and the authors noted that not all facilities used sporicidal products and 6 of the hospitals used neutral cleaner for patient rooms when the patient was not under contact precautions. Thus environmental dissemination from asymptomatic patients cannot be ruled out. Even including this reservation, the Mitchell (2019) study provides another example of a large multi-hospital study where a moderate improvement in cleaning compliance did not improve CDI rates, further demonstrating the limited associated between environmental contamination and CDI rates.

Boyce (2017) showed an impact on reducing on a composite *C. difficile* colonization and infection rate in a study where an improved hydrogen peroxide disinfectant was used instead of a chlorine disinfectant for daily and discharge cleaning of rooms with patients with CDI and cleaning compliance was maintained at >80%. Incident densities for *C.*

difficile decreased from 1.0 per 1000 patient days to 0.56 per 1000 patient days. The study reported a composite incidence density that included MRSA and VRE, which showed a 23% reduction, but this did not achieve statistical significance and individual rate reductions were not assessed for statistical significance.

The challenges in cleaning patients' rooms to remove all *C. difficile* was demonstrated by Sitzlar (2013), who executed three different interventions in a stepped-wedge design (use of fluorescent marker for discharge cleaning, addition of UV-C unit on discharge cleaning and use of fluorescent marker for daily cleaning and use of a dedicated cleaning team for cleaning (daily or discharge) for all CDI patient rooms). While each step of the intervention produced some improvement, only the dedicated cleaning team was able to consistently remove all *C. difficile* from environmental samples (Sitzlar, 2013). This study demonstrated that if removal of all *C. difficile* from the environment was a necessary requirement to reduce the risk of CDI rates, this would be challenging to do with current cleaning staff given the challenges in consistently and correctly cleaning a CDI patient room even when training and cleaning validation are performed.

Rutala (2012) demonstrated that removing low numbers of *C. difficile* spores from a surface is not particularly challenging and that 5 different cleaning methods tested which involved physically wiping the surface removed at least 2.90 $\log_{10}$ of *C. difficile* spores, including a nonwoven disposable wipe impregnated with a non-sporicidal quaternary disinfectant. Weber (2013) reviewed several studies investigating the level of contamination of environmental surfaces with *C. difficile* spores and reported that typically <1 log/cm^2 of spores were found in the environment. In the papers reviewed, the largest number of spores on a surface was 1,300 colonies when a sponge collection method was used. (Weber 2013) This work aligns with the results from the Sitzlar (2013) study and suggests that absent visible soil, any surface with low levels of *C. difficile* spores (as would commonly be found in a clinical setting) would likely find the spores removed through the physical wiping of the surface. It suggests that *C. difficile* spores found in a patient room after cleaning are more likely to be the result of a failure to wipe the surface than wiping the surface and failing to remove the *C. difficile* spores.

In an attempt to improve on cleaning compliance, many facilities have implemented so called "no touch" disinfection technologies such as fogging with hydrogen peroxide or the use of portable UV-C units. While a review of these technologies is beyond the scope of this paper, we will briefly discuss the largest of these studies. Anderson (2017) conducted a 9-hospital study using four different patient room discharge cleaning methods, including quaternary disinfectant (the reference practice), quaternary disinfectant followed by UV-C, bleach, and bleach followed by UV-C, for all patient rooms where there was a patient on contact precautions. Rooms with patients having CDI did not use the quaternary disinfectant and were only cleaned with bleach or bleach followed by UV-C. The rate of CDI showed a non-significant increase during the study period when UV was added. While UV-C has been shown to reduce environmental contamination of *C. difficile*, and in the Anderson (2017) study swabbed surfaces showed the *C. difficile* contamination

declined when UV-C was added to the cleaning procedure, this decrease in contamination did not impact *C. difficile* rates, suggesting either that environmental exposure to *C. difficile* plays a minor role in subsequent infection for other patients or that the level reduction was not significant enough to decrease CDI rates to a level of statistical significance. As the UV-C unit used in the study was not used in the toilet area and CDI patients are known to contaminate the environment when flushing the toilet (Wilson, 2020), it is also possible the testing for *C. difficile* spores did not test in areas of high contamination and that the UV-C unit had minimal impact on an area of high contamination.

One of the more thorough reviews of the literature was used for the current SHEA/IDSA guidance document on strategies for patients with CDI. The document discusses the mixed evidence on the role of the environment for CDI transmission and recommends the use of sporicidal products in outbreak or hyperendemic settings but does not recommend the use of a sporicidal disinfectant for all CDI patients and grades this recommendation as having very low-quality evidence (Dubberke, 2014). The comparable European recommendations from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) similarly recommends sporicidal disinfectants in outbreak or high endemic rate facilities, but also grades the evidence supporting this as having very low quality of evidence (Tschudin-Sutter, 2018).

Peterson (2020) reported on a 12-month study in 4 hospitals where a bundle of improved infection prevention practices (bleach cleaning, hand hygiene with soap and water, hand hygiene compliance, proper use of PPE, use of a portable UV-C unit, measuring cleaning compliance, testing for colonization on admission, and monitoring of physician ordered *C. difficile* testing) were tested for their ability to lower CDI rates. Hand hygiene compliance, room cleaning practices, the use of the portable UV-C unit, and proper PPE usage appeared to have no impact on CDI rates (Peterson, 2020). Targeted admission screening for colonization paired with appropriate infection prevention practices reduced CDI rates to a low level during the study (Peterson, 2020).

Doll (2020) implemented a series of interventions to reduce the rate of HO-CDI including 2 step cleaning in CDI rooms, a UV-C device, contact precautions plus handwashing with soap and water, use of a sporicidal disinfectant for all patient rooms, and using the electronic medical record (EMR) assistance tool to guide physicians in ordering *C. difficile* testing, all of which were audited for compliance. The only intervention that decreased the HO-CDI rate was the EMR guided testing protocol, which led to lower CDI rates, but with fewer tests being done overall. While it was not a study goal to reduce the amount of *C. difficile* testing, it was a result of the new testing protocol and the authors stated this confounds interpreting the results.

Chau (2020) performed a systematic review of studies that included an environmental cleaning bundle as part of interventions to reduce CDI and 10 studies were included in the final review. While the interventions improved cleaning compliance, such as by increasing the removal of a fluorescent marker (RR=1.55) and reducing the number of rooms that were positive for *C. difficile*

(RR=0.16), they did not have a statistically significant effect on the HO-CDI rate (RR=0.96).

Genetic Testing and Impact on Classification of *C. difficile* Transmission

A number of studies have claimed a strong link between contact with colonized or infected patients and the subsequent risk of a patient developing CDI. Limits in the design of these studies has made proving this link through the existing literature challenging. Recent studies that have combined hospital admissions data and genotyping have shown that transmission through hospital-based contact with patients with active CDI generally accounts for less than 25% of subsequent cases, but these studies have not always accurately investigated the role of colonized patients (Eyre, 2013) and some studies have reported higher levels of transmission related to hospital level contacts, suggesting a wide range may be possible depending on facility specific characteristics. Importantly, studies that use multilocus sequencing or ribotyping are limited by the probability that a large number of patients will share the same ribotype, but not the same genetic strain (Eyre, 2013).

A study by Kociolek (2018) studied CDI in symptomatic children and found that of 107 children with 131 CDIs, Whole Genome Sequencing (WGS) identified 104 genetically distinct isolates with only 8 of the isolates present in more than one patient. Further, of the 8 isolates present in more than one patient, 2 of the isolates were present in 3 patients and none infected more than 3 patients. This data suggests that indirect transmission between children with CDI occurred in ~12% of cases, which is less than reported for adults and the variation in strains is substantial, indicating a large environmental reservoir for *C. difficile*. While patient to patient transmission is still an important potential vector, the frequency with which this occurs may be significantly less than currently is assumed outside of outbreak conditions, where the risk may be higher. The authors (Kociolek, 2018) comment that their strict adherence to infection prevention practices and proactive placement of all patients with diarrhea in contact precautions may be helping to control the risk of patient-to-patient transmission, keeping incidence rates below what other hospitals experience.

Widmer (2017) studied 750 patients with toxigenic *C. difficile* (incidence of 2.88 patients per 10,000 patient days). These patients came in contact with 451 patients, 27 of whom (6.0%) developed CDI, indicating possible transmission within the hospital (Widmer, 2017). The 6% potential transmission rate is lower than in several other studies, but this study was done in Switzerland and may reflect local hospital conditions which are different from other countries. Ribotyping and studying of potential contact between the patients studied indicated possible transmission in only 6 of 27 contacts, which suggests an overall potential for patient-to-patient transmission of only 1.3% of the at risk patients (6 of 451) (Widmer, 2017). CDI incidence increased from 2.8 to 4.3 cases per 10,000 patient days during the study. Limited environmental testing was done for 16 patients and 2.3% of samples (3 of 128) were positive for toxigenic *C. difficile*, all of which were toilet seats, reinforcing the importance of dedicated toilets for patients with CDI as part of controlling environmental dissemination. This study provides additional evidence that a small portion of *C. difficile* transmission

likely occurs in acute care and most of the exposure resulting in colonization or infection is likely to occur in other settings.

Babady (2021) used MLST to test patient samples from 1,365 patients in a hospital over a 2 year period with 542 community cases (potentially CA-CDI), 400 hospital onset cases (potentially HO-CDI), and 383 post-discharge cases (potentially HO-CDI). They found that only 8.3% of the post discharge cases had contact with a patient with a similar MLST strain and the authors concluded that symptomatic CDI patients and environmental contamination are generally not the source of infection for other patients on the same unit that develop CDI post-discharge, but post-discharge antibiotic exposure should be considered during surveillance and stewardship efforts.

Summary

This paper discussed evidence of *C. difficile* transmission through potential exposure within a healthcare environment, the risk of colonization through other potential environmental exposures, the prevalence of environmental shedding from colonized and infected patients, whether healthcare workers are likely to transmit *C. difficile* to patients, whether colonized or infected patients are likely to transmit *C. difficile* to other patients, the impact improved cleaning and disinfection programs have on CDI rates, and how better molecular epidemiological methods, such as whole genome sequencing (WGS), are providing new tools to better understand whether patient to patient transmission occurs in a healthcare facility.

Emerging evidence suggests that routine environmental exposure to *C. difficile* is responsible for the majority of colonization and while healthcare transmission does occur, it is likely a minority of the transmission, perhaps 10-20% of CDI cases.

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