Curr Infect Dis Rep (2015) 17: 43
DOI 10.1007/s11908-015-0499-0

The Importance of Colonization with *Clostridium difficile* on Infection and Transmission

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Published online: 4 August 2015

Abstract *Clostridium difficile* infections (CDI) are the most common cause of healthcare-associated infections (HAI) in the USA, accounting for 12 % of all HAIs [1]. Reasons for such an increase are unknown but may relate to antibiotic use and evolution of a new, pathogenic strain, NAP1/BI/027. The Centers for Disease Control and Prevention (CDC) identifies *C. difficile* as one of only three organisms to be assigned a designation of an "urgent" threat level. Asymptomatic colonization with *C. difficile* is much more common than symptomatic CDI and has been documented to contribute to new cases of CDI. Despite this knowledge, approaches to managing and preventing transmission from asymptomatically colonized patients are lacking. Enhanced cleaning, avoidance of unnecessary antimicrobials, and use of gowns and gloves for patients with CDI are the cornerstone of *C. difficile* management in patients with known disease. Infection control interventions to prevent transmission from asymptomatically colonized patients have not been determined.

Keywords *Clostridium difficile* infections · CDI · Healthcare-associated infections · HAI

This article is part of the Topical Collection on *Healthcare Associated Infections*

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Introduction

Clostridium difficile infection (CDI) is the most common cause of healthcare-associated infections (HAI) in the USA, accounting for 12 % of all HAIs [1]. The number of patients in the USA with CDI on discharge (based on medical coding) doubled from 2001 to 2010 [2, 3]. According to the data from the CDC's Emerging Infections Program, among epidemiologically significant pathogens, *C. difficile* has by far the highest population-based incidence, estimated at 147 per 100,000 compared to 25 for methicillin-resistant *Staphylococcus aureus* (MRSA) and 3 for carbapenem-resistant *Enterobacteriaceae* (CRE) [4–7]. The Centers for Disease Control and Prevention (CDC) has identified CDI as one of the three most important or "urgent" threats to public health. Despite the growing awareness of CDI, we have limited understanding of the relationship between colonization with toxigenic *C. difficile* and subsequent CDI, the contribution of asymptomatic carriers to transmission, and methods to prevent CDI [8, 9]. In this manuscript, we will review literature to summarize the current understanding of colonization with toxigenic *C. difficile* and its association with subsequent infection and patient-to-patient transmission in the acute care setting.

Epidemiology

Asymptomatic *C. difficile* colonization was first studied in the 1980s after the organism was initially found to be associated with antibiotic-associated diarrhea [10]. Interest in *C. difficile* epidemiology was renewed with the emergence of the virulent NAP1/BI/027 strain in North America and Europe in the early 2000s [11]. As control over the spread of *C. difficile* remains poor with steady or increasing rates across the USA in the last decade, further investigation into the role of asymptomatic

carriers continues, utilizing newer molecular methods for both detection and strain typing. To date, although asymptomatic colonization with toxigenic *C. difficile* has been found across the spectrum of healthcare facilities, the role of asymptomatic carriers in transmission of this organism is not fully understood and no major infection prevention guidelines offer specific recommendations for identifying or targeting asymptomatically colonized persons. Frequency of asymptomatic colonization with both toxigenic and non-toxigenic strains has been examined by many authors. A recent meta-analysis reviewed frequency of colonization at hospital admission from 1990 to 2014 with the most recent studies from the USA identifying 7.5–15.5 % of patients asymptomatically colonized with toxigenic *C. difficile* at admission [9]. Older US studies also indicate that the prevalence of colonization with *C. difficile* increases to approximately 20 % over the course of hospitalization [12, 13]. In contrast, active CDI is estimated to occur in approximately 1.4 % of hospitalized patients in the USA (Fig. 1) [1].

Relationship of Genotype with Colonization vs. Infection

It is unclear if the specific strain type of *C. difficile* is related to propensity to cause CDI vs. asymptomatic colonization. Observational studies have suggested that CDI is more often associated with the NAP1/BI/027 strain than asymptomatic colonization. In one cohort, healthcare-associated CDI was more frequently associated with the NAP1/BI/027 strain (52/83 cases [63 %]) compared to both healthcare-associated asymptomatic colonization (43/119 cases [36 %]) and colonization on admission (24/181 [13 %]) [14]. In a separate study, the NAP1/BI/027 strain was found in approximately 25 % of cases of CDI vs. 3 % of patients with asymptomatic colonization on admission to the hospital [15]. Didelot et al. recently used whole genome sequencing to assess the relatedness of strains of epidemiologically linked pairs and found that while overall only 19 % of pairs shared a common ancestor (implying transmission), this proportion was much higher (63 %) for the NAP1/BI/027 strain [16].

Diagnosis and Treatment

Although there are now more diagnostic assays, including nucleic acid detection-based methods, to test for *C. difficile*, clinical decision-making is required to avoid overdiagnosis and overtreatment of colonization without disease [8]. CDI is a clinical diagnosis; laboratory findings can support but not confirm a diagnosis. No laboratory test can distinguish between asymptomatic colonization and infection although the detection of free toxin in stool, when available, is more specific for CDI (vs. asymptomatic colonization) [8]. As noted in the figure, the majority of hospitalized patients with *C. difficile* are asymptomatically colonized. Indiscriminate testing of patients with brief episodes of diarrhea or patients with other causes of diarrhea such as laxatives will lead to false positives (overdiagnosis) for CDI and the use of additional unnecessary antibiotics. Antibiotic therapy for CDI carries a paradoxical increased risk of causing CDI once it is stopped. Therefore, education and protocols are essential to assure testing occurs only in patients with a clinical syndrome for CDI.

The most common assays used by clinical microbiology laboratories in the USA to detect *C. difficile* in stool are nucleic acid amplification tests (NAAT) [8]. While the reported analytical performance characteristics of these assays vary depending upon the gold standard used for comparison, they have a sensitivity of 86–92 % and specificity of 94–97 % [8]. However, this specificity is for detection of toxigenic *C. difficile* in stool, not CDI, as the studies did take into account the entire clinical picture. The specificity of NAATs for CDI is more likely in the mid-80 % range [17]. Other approaches to testing include toxin testing and multistep algorithms and are reviewed elsewhere [8, 18].

Treatment for CDI has generally been based on oral metronidazole for mild to moderate disease and oral vancomycin for more severe cases, but more recent data indicate metronidazole is inferior to vancomycin even for mild to moderate disease [8, 19]. Fidaxomicin became only the second FDA-approved treatment for CDI in 2011 (the other FDA-approved treatment is oral vancomycin). Fidaxomicin was non-inferior to vancomycin for initial treatment response (88.2 vs. 85.8 %) but was associated with a statistically significant reduction in recurrent CDI (15.4 vs. 25.3 %), presumably due to less microbiome disruption [20]. Multiple recurrent CDI (three or more CDI episodes) is a major clinical challenge. Use of fecal microbiota transplantation has gained in popularity. Recent studies indicate an efficacy of 70 to 80 % to prevent additional recurrences with a single fecal transplant [21]. No therapies are indicated for colonization without CDI [22].

Presence of asymptomatic colonization may increase risk for CDI. In the 1990s to 2000s, prior to the emergence of the epidemic strain, the predominant thinking was that while *C. difficile* was a necessary step towards infection, prolonged colonization was ultimately protective against active disease [23, 24]. The mechanism of this protective effect has not been fully elucidated but boosting of serum antibody levels in those colonized over long periods of time is thought to play a role [24, 25]. The protective effects could also be due to the overall composition of the microbiota when *C. difficile* is a component of the community. More recently, patients with *C. difficile* colonization after resolved CDI who are subsequently exposed to antibiotics have an approximately one in four chances of developing CDI [26]. Furthermore, recent data from a systematic review and meta-analysis suggests that patients with toxigenic *C. difficile* colonization are six times more likely to develop CDI than non-colonized patients [9].

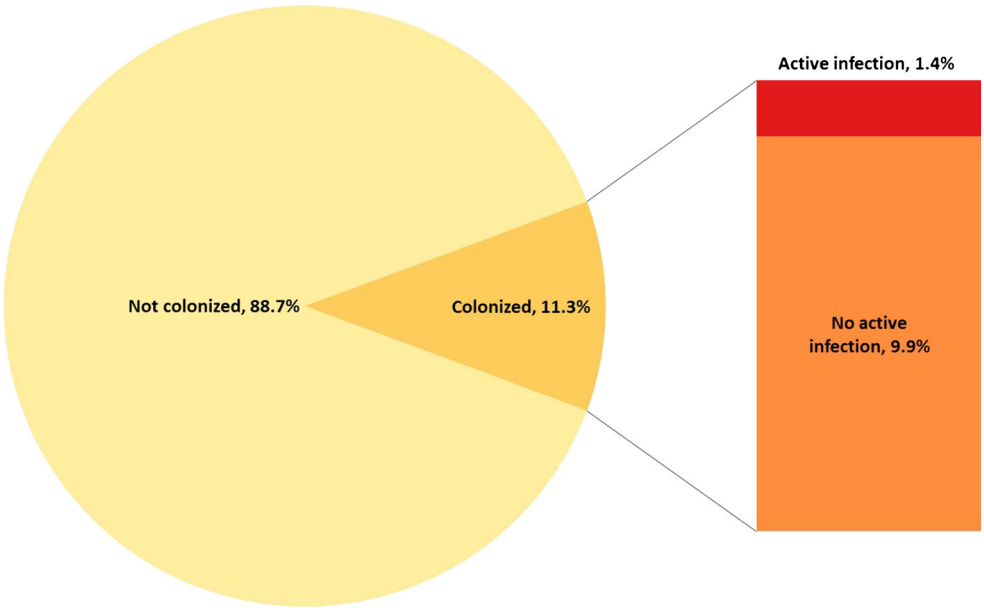

Fig. 1 Demonstrating relationship between asymptomatically colonized patients and those with active *C. difficile* infection (*CDI*). The entire hospital population is represented in the *circle* and the 11.3 % with *C. difficile* in the *darker wedge*. Notably, approximately 1.4 % of patients have disease while 9.9 % have asymptomatic colonization [1, 9]

While this contradicts previous studies of colonization being protective against disease, several limitations and alternative explanations for the findings of that systematic review need to be considered: (1) A low threshold for testing coupled with the high sensitivity of NAAT assays for *C. difficile* results in a high probability of a positive test among patients with *C. difficile* colonization and diarrhea from other causes, biasing towards a higher incidence of "CDI" in these patients; (2) the total number of CDI cases among people not colonized on admission was twice the number of CDI cases among colonized patients, and attack rates in colonized patients (10 %) were lower than the attack rates seen after a new *C. difficile* acquisition in a hospitalized patient (30–50 %); (3) initial colonization is a necessary precursor of infection, and most people not colonized on admission do not acquire *C. difficile*. Therefore, the question is not whether colonized patients are at risk for CDI but how that risk changes with the duration of colonization, and whether a duration "threshold" exists beyond which colonization becomes protective.

Asymptomatic Colonization as Source for C. Difficile Infection

In one of the first studies to assess the contribution of asymptomatic and symptomatic patients to in-hospital *C. difficile* transmission using restriction enzyme analysis (REA) typing to distinguish between strains, Clabots et al. found that nosocomial acquisition of a *C. difficile* strain was preceded by introduction of that strain to the ward by an asymptomatic admission in 84 % of cases [12]. More recently, Curry et al. used multilocus variable number of tandem repeats analysis for assessing relatedness of isolates in a study using PCR to detect *C. difficile* from peri-rectal swabs from asymptomatic patients and stool from patients with CDI [27]. Among 56 incident cases of hospital-acquired CDI with available isolates, 17 (30 %) cases were associated with patients with CDI, whereas 16 (29 %) cases were associated with asymptomatic carriers. Of note, only ~25 % of people were screened for asymptomatic *C. difficile* carriage, so many of the 41 % of CDI cases that were not directly linked to another CDI case or asymptomatic carrier could have been the result of transmission from an asymptomatic carrier [28]. In a study using whole genome sequencing of 1223 of 1250 cases with symptomatic CDI in either healthcare or community settings in Oxfordshire, UK, Eyre et al. found that 45 % of cases were genetically distinct from all previous hospital or community cases, pointing towards the existence of an asymptomatic or community reservoir [29]. The limitations of this study were the inclusion of only toxin-positive CDI cases detected by enzyme immunoassay (EIA), which could have led to exclusion of a large proportion of cases because of a lower sensitivity of toxin EIAs, and the lack of screening for asymptomatically colonized persons. The same investigators also conducted a small prospective study to assess the potential for transmission from asymptomatically colonized patients [30]. In this study, stool cultures were performed on 132 of 227 patients in two UK hospitals, of which 14 (11 %) were positive on the initial sample and 18 overall. Two patients on the same ward were found to be asymptomatically colonized with similar isolates—this was thought to either represent transmission from one asymptomatic patient to another or both having been exposed to a common source that had not been cultured. While transmission from asymptomatically colonized patients was not a frequent occurrence in this study, findings are greatly limited by a small sample size and incomplete data on colonization status. In one review of multiple studies before

2001, 15 % of patients asymptomatically acquired *C. difficile* [31].

Asymptomatic Colonization and Environmental Contamination

Contamination of healthcare worker's (HCW) hands, attire, or the healthcare environment of *C. difficile*-colonized patients has also been assessed. Kim et al. cultured toxigenic *C. difficile* from floors and other surfaces in rooms of 9 % of *C. difficile* carriers with diarrhea and 3 % of similar sites surrounding asymptomatic carriers (which may be lower, based on older culture methods than more recent studies) [32]. Similarly, McFarland et al. found that room contamination occurred in 49 % of symptomatic compared to 29 % of asymptomatic *C. difficile* cases, and cultured *C. difficile* from the hands of 59 % of HCWs caring for symptomatic or asymptomatic patients [33]. Both of these studies found less, but significant, environmental contamination associated with asymptomatic carriers compared to patients with CDI and diarrhea. Among residents of a long-term care facility in the setting of a *C. difficile* outbreak, Riggs et al. found that 51 % patients were asymptomatically colonized [34]. Asymptomatic *C. difficile* colonization was associated with skin and environmental contamination; 87 % of isolates from patient skin samples and 59 % of environmental samples had isolates identical to the patient's stool *C. difficile* isolate [34]. Transfer of organism to investigator's hands occurred in 58 % of cases. Interestingly, this study found skin and environmental contamination among patients even in the absence of *C. difficile* colonization or infection. This, in conjunction with the finding that 41 % of the environmental strains did not match the strain colonizing the current occupant, also suggested ongoing contamination from prior occupants who had either colonization or disease [34]. Other studies have similarly shown frequent environmental contamination [35]. In another recent point-prevalence study of asymptomatic colonization in an acute care hospital, 18 of 149 (12 %) patients were carriers of toxigenic *C. difficile* [36]. Similar to previous findings, skin and/or environmental contamination was lower in asymptomatic carriers (3/18, 17 %) compared to symptomatic CDI patients (5/6, 83 %; P=0.007).

Colonization of Healthcare Workers

Healthcare workers can be colonized with *C. difficile*. Studies estimate that approximately 0–5 % of HCW in hospitals are colonized with *C. difficile*, no different than that seen in healthy, community-dwelling adults [32, 37, 38]. In their investigation of the environment and contacts with patients after a single CDI case on a pediatric ward, Kim et al. cultured hands and stool of HCWs working on the ward [32]. They recovered toxigenic *C. difficile* from 2 of 12 samples from HCW hands and from the stool of 2 of 11 asymptomatic nurses working on the ward. In an early study at an academic hospital in the USA, stool cultures were performed among 55 HCWs with direct contact with patients in wards with an estimated *C. difficile* prevalence of 3.8 % and no HCWs were found positive [37]. More recently, in a large point-prevalence study among HCWs at a large teaching hospital in Australia, in a non-outbreak setting, stool *C. difficile* colonization was assessed using enzyme immunoassay followed by culture among those with a positive EIA test (which would likely underestimate colonization compared to culture or PCR) [39]. Of 128 HCWs in that study, 41 % reported recent contact with a patient with known or suspected CDI and none were found to be colonized with *C. difficile* [39]. Similarly, in another recent non-outbreak setting at a university hospital in Sweden which used cultures of rectal swabs, the colonization rate among HCWs was 0 % (0/22) and was associated with a patient admission prevalence of 5.2 % (3/58) [38]. These studies suggest that HCW colonization in non-outbreak settings is no different from healthy individuals in the general population [40, 41].

Infection Control Considerations and Colonization

C. difficile prevention recommendations are divided into recommendations for all hospital settings, and an added level of prevention strategies for hospitals with ongoing problems with *C. difficile* where basic recommendations are insufficient for control (see Table 1) [22]. Current infection control recommendations for *C. difficile* are directed at patients with CDI, with guidelines recommending similar precautions for patients after resolution of CDI during the same hospitalization for hospitals with a high incidence of *C. difficile* [22] on the basis of prolonged shedding of *C. difficile* spores after a recovery from an active disease [42]. Use of contact precautions (gowns and gloves) is recommended for contact or room entry of patients known to have CDI. Some have advocated use of empiric contact precautions for all patients with diarrhea based on the recognition that *C. difficile* and norovirus are the primary infectious causes of diarrhea and both are managed with contact precautions [43, 44]. Also, if a hospital is having difficulty controlling CDI despite proper cleaning of the environment, it is recommended to consider using a sporicidal disinfectant to enhance removal of viable *C. difficile* spores [22].

Studies of Interventions Targeting Asymptomatically Colonized Persons

To date, no studies have evaluated the impact of active surveillance for *C. difficile* or measures to limit *C. difficile* transmission from asymptomatically colonized persons. Modeling studies have estimated mixed results with active surveillance

Table 1 Infection control interventions for *C. difficile* and their application for colonization vs. infection with *C. difficile*

Patient population applied to	Hand hygiene with soap and water (not alcohol hand sanitizer)	Room cleaning with sporicidal agent	Contact precautions	Lab-based alert system	Antimicrobial stewardship	Universal gown and glove use[a]
Asymptomatic colonization with C. difficile	No	No	No	No	Yes	Yes
C. difficile infection (CDI)	Yes	Yes	Yes	Yes	Yes	Yes
Post-CDI, same hospitalization	variable[b]	Variable[b]	Variable[b]	No	Yes	Yes

[a] Glove and gown use for all patients in a ward or unit, independent of *C. difficile* status

[b] Variable and generally recommended for ongoing problems with *C. difficile* despite use of basic measures

due to poor PCR sensitivity for *C. difficile* in asymptomatically colonized patients and prolonged turnaround time for culture [45]. Suggestions from the literature for enhanced *C. difficile* prevention include active surveillance culturing with isolation [46], prolonged isolation of patients with past CDI who are presumed to be colonized (and potentially, enhanced stewardship to avoid antibiotic use) [46], enhanced environmental cleaning of patients known to be asymptomatically colonized with *C. difficile*, decolonization if a regimen to safely decolonize patients without paradoxically increasing the risk of CDI once stopped could be identified [46] or treatment with probiotics such as non-toxigenic *C. difficile* [47].

Given limitations in detection, universal approaches may be more feasible for asymptomatically colonized patients. In a single-center study using bleach wipes for daily cleaning of all patient rooms (regardless of CDI or colonization status) in two wards with high endemic rates of CDI, Orenstein et al. found "an 85 % decrease in hospital-acquired CDI over a 12-month period, and the median time between hospital-acquired CDI cases" increased from 8 to 80 days [48]. However, this was not compared to a strategy of using bleach only in rooms occupied by patients with CDI and may have reflected regression to the mean given the high CDI rates at study onset.

In summary, the *C. difficile* epidemic has continued despite aggressive environmental cleaning in many facilities. Methods to address transmission from patients with asymptomatic *C. difficile* colonization have not been defined although multiple approaches have been proposed.

Conclusions

Clostridium difficile is increasing in frequency worldwide. Reasons for such an increase are unknown but may relate to antibiotic use and evolution of new strain types with increased pathogenicity and antimicrobial resistance determinants, such as the NAP1/BI/027 strain. The CDC identifies *C. difficile* as one of only three organisms at an "urgent" threat level. Although asymptomatic colonization with *C. difficile* is much more common than active CDI and asymptomatic colonization has been documented as a source of new cases of CDI, approaches to managing and preventing transmission from asymptomatically colonized patients are lacking. Enhanced cleaning, careful avoidance of antimicrobials, and use of gowns and gloves for patients with CDI are the cornerstone of *C. difficile* control in patients with known disease. The appropriate precautions for patients with asymptomatic colonization are unknown.

Compliance with Ethics Guidelines

Conflict of Interest Carey-Ann Burnham reports grants from Cepheid, bioMerieux and Accelerate Diagnostics. Erik Dubberke reports grants and personal fees from Sanofi Pasteur and Merck. Surbhi Leekha received honorarium from the American Hospital Association/Health Research and Educational trust. Daniel Morgan reports personal fees from Welch Allyn, grants from VA HSRD, other from IDSA, ASM, and SHEA for expenses to organize or present at national meetings and personal fees from 3M.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by the author.

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