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Original Research Article

IDENTIFICATION OF CLOSTRIDIUM DIFFICILE ASYMPTOMATIC CARRIERS AMONG HEALTH CARE WORKERS IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: *Clostridium difficile* (*Clostridioides difficile*) infection (CDI) is a significant healthcare-associated infection that causes antibiotic-associated diarrhea and has a substantial impact on morbidity worldwide. Asymptomatic carriers among healthcare workers (HCWs) may serve as potential reservoirs for transmission within hospital settings. However, data regarding asymptomatic carriage among HCWs in India remain limited. **Objectives**: To determine the prevalence of asymptomatic *Clostridium difficile* colonization among healthcare workers in a tertiary care hospital in Coimbatore, Tamil Nadu.
Materials and Methods: An analytical cross-sectional study was conducted over two months among healthcare workers in a tertiary care hospital. A total of 124 HCWs consented to participate, of whom 100 provided stool samples for laboratory analysis. Fresh stool specimens were screened for *C. difficile* glutamate dehydrogenase (GDH) antigen using the VIDAS GDH assay. GDH-positive samples were further tested for toxins A and B using the VIDAS. *C. difficile* toxin A/B enzyme-linked fluorescence immunoassay.
Results: Among the 100 participants, 73% were females, of whom around 76% were nursing staff. Ten percent of participants reported occupational exposure to patients with chronic diarrhea or gastrointestinal infections. Four stool samples (4%) tested positive for GDH antigen; however, all GDH-positive samples were negative for toxins A and B. No evidence of active toxigenic *C. difficile* infection was identified among the study population. The GDH-positive but toxin-negative findings suggest possible asymptomatic colonization or carriage of non-toxigenic C. difficile strains.
Conclusion: The present study demonstrated a low prevalence (4%) of asymptomatic *C. difficile* colonization among healthcare workers in a tertiary care hospital setting. Although no toxigenic strains were detected, asymptomatic carriers may contribute to hospital-associated transmission. Periodic surveillance, infection control practices, antimicrobial stewardship, and increased awareness among healthcare workers are essential to prevent the emergence and spread of CDI in healthcare settings.
Keywords: *Clostridium difficile*, asymptomatic carriers, healthcare workers, GDH antigen, hospital-acquired infection, colonization.

INTRODUCTION

The human gastrointestinal tract is divided into sections, allowing digestion and nutrient absorption in the proximal region to be separate from the vast microbial populations in the large intestine. In normal adults, the esophagus contains microorganisms arriving with saliva and food. The stomach's acidity keeps the number of microorganisms to a minimum unless pyloric obstruction favours the proliferation of Gram-positive cocci and bacilli. In a normal adult colon, 96-99% of the resident bacterial flora consists of anaerobes.[1-3]

The important functions of intestinal microbiota can be divided into three major categories. The first of these is the protective function, in which resident bacteria indirectly displace and inhibit potential pathogens by competing for nutrients and receptors, or directly by producing antimicrobial factors such as bacteriocins and lactic acid. Second, commensal organisms are important for the development and function of the mucosal immune system. They induce IgA secretion, influence the development of the intestinal humoral immune system, and modulate local T-cell responses and cytokine profiles. The third category consists of a broad range of metabolic functions. The microbiota of the small intestine can contribute to the host's amino acid requirements when the diet does not provide these. Intestinal bacteria produce short-chain fatty acids that control intestinal epithelial cell differentiation.[1-4]

The genus Clostridium is extremely heterogeneous, and more than 200 species have been described; its members are ubiquitous in the environment and transmitted to humans by ingestion. The list of pathogenic organisms, as well as novel species isolated from human faeces whose pathogenic potential remains undetermined, continues to grow. Clostridia cause several important toxin-mediated diseases, including tetanus (C. tetani), botulism (C. botulinum), gas gangrene (C. perfringens), and antibiotic-associated diarrhoea and pseudomembranous colitis (C. difficile).

Clostridium difficile is the major cause of hospital-acquired (nosocomial) diarrhoea. Clostridium difficile (C. difficile) is a Gram-positive, spore-forming, anaerobic bacillus.[1] It is part of the normal flora of the gastrointestinal tract and is present in 1-3% of healthy adults, with higher rates of colonization in infants.[2] C. difficile is recognized as one of the most important pathogens in hospital and community healthcare settings, with a steadily rising global incidence of infection and concordant increase in mortality.[3,4] The Centre for Disease Control and Prevention (CDC) has assigned C. difficile as an urgent threat because of its association with antibiotic use, high mortality, and morbidity.[5] The clinical spectrum of symptomatic C. difficile infection (CDI) ranges from mild diarrhoea to severe complications such as pseudomembranous colitis, toxic megacolon, bowel perforation, sepsis, and death.[6] It is mediated through the production of toxins (toxins A and B).[7] An additional putative virulence factor, the binary toxin, is produced by some strains, particularly the more virulent epidemic strains such as BI/NAP1/027, and may also be present in the absence of toxin A or toxin B.[8]

Pseudomembranous colitis is diagnosed by the detection of one or both C. difficile toxins in stool and by endoscopic observation of pseudomembranes or microabscesses in patients who have diarrhoea and have been given antibiotics. Plaques and microabscesses may be localized to one area of the bowel.[5-8]

Antimicrobial drugs taken orally can temporarily suppress the drug-susceptible components of the faecal flora in humans. The acute effects of antibiotic treatment on the native gut microbiota range from self-limiting diarrhoea to life-threatening pseudomembranous colitis. However, soon thereafter, the counts of faecal flora rise again to normal or higher-than-normal levels, principally of organisms selected out because of their relative resistance to the drugs used.

Asymptomatic C. difficile colonization remains a complex and challenging health problem because its epidemiological features vary considerably across study groups and settings. Asymptomatic C. difficile colonization is a condition where C. difficile is detected in the absence of symptoms of infection.[9] It has been proposed that asymptomatic C. difficile colonized patients may be protected from progression to infection because they can mount a humoral immune response to clostridial toxins. However, asymptomatic C. difficile-colonized patients may act as an infection reservoir and pose a risk to others.[10] The number of colonized patients is higher than symptomatic CDI cases among hospital patients, particularly when the disease is endemic.[11] Estimates indicate that approximately 20.0% of hospitalized adults are C. difficile carriers who shed the bacterium in their stool.[12] The prevalence of asymptomatic C. difficile colonization varies depending on several host, pathogen, and environmental factors – making colonized patients a potential vehicle of transmission of C. difficile in healthcare environments, particularly with the global spread of emergent hypervirulent toxigenic strains. Few studies have synthesized evidence on the role and importance of asymptomatic C. difficile colonization in the progression to symptomatic CDI, the transmission of infection, or the challenges to CDI control.[13,14]

The carriage of C. difficile by healthcare workers (HCWs) has been studied previously with conflicting results. Carmelli et al,[15] and Hell et al,[16] cultured the stool of healthcare workers in a non-outbreak period and found that none of the specimens contained C. difficile. However, studies reported from Japan and the Netherlands have found 4.3% and 13.0% of HCWs, respectively, colonized with C. difficile.[17] The Centre for Disease Control and Prevention (CDC) classified CDI as an urgent public health threat requiring immediate surveillance and control measures in 2013.

The evidence generated by this research can inform policy decisions about periodic follow-up of asymptomatic C. difficile carriers among healthcare workers. The findings will also guide further research on the optimal testing interval for healthcare workers for C. difficile carrier status. With these in mind, the primary objective of the present study was to estimate the point prevalence of Clostridium difficile stool colonization among healthcare workers (HCWs) in a tertiary care hospital in the Coimbatore district, Tamil Nadu.

MATERIALS AND METHODS

Study design and population

This was an analytical cross-sectional study conducted over two months among healthcare workers in a tertiary care hospital setting in Coimbatore district, Tamil Nadu.

Sample size and justification

In previous reports of intestinal colonization of healthy adults by *C. difficile*, colonization rates were as high as 17.5%.[18] Considering a 17.5% proportion of asymptomatic healthcare workers with intestinal colonization by C. difficile and an absolute precision of 10.0%, the computed sample size was 56. However, after accounting for a 10.0% non-response rate, the estimated minimum sample size was 62 asymptomatic healthcare workers with 95% confidence. However, in the present study, we planned to include 100 asymptomatic healthcare workers.

Inclusion / Exclusion criteria

Inclusion Criteria

Healthcare workers are defined as "health care service providers and other workers in health care settings, including nursing staff, medical officers, paramedical staff, and supporting staff like sanitation workers.

Exclusion Criteria

- Participants with a history of diarrhea.
- Participants are unwilling to provide informed written consent.

Selection of Study Subjects and Data Collection

The study was initiated after approval from the Institutional Ethics Committee (IEC) (Ref No: 228/2023). The Participant Information Sheet (PIS) in the local language was provided to the study subjects, and its contents were read to them in their own language to their satisfaction.

The study subjects were selected through simple random sampling and subsequently enrolled after written informed consent was obtained. Data will be collected using a purpose-designed, structured, and pre-tested questionnaire.

Clinical Sample Collection

The samples (stool samples) will be collected in a clean, dry disposable container. Only freshly taken specimens will be processed, and if the tests cannot be performed immediately, they will be kept at 4°C–8°C until processing.

Detection of Clostridium difficile

The detection of *C. difficile* was done using a commercially available *Clostridium difficile* detection kit - VIDAS *C. difficile* GDH and VIDAS *C. difficile* toxin A/B (Enzyme-linked fluorescence immunoassay) for the detection of *C. difficile* glutamate dehydrogenase (GDH) antigen and toxins A and B using specific antibodies in a separate testing reaction well. Following the manufacturer's instructions, the tests will be performed and the results interpreted.

Each stool specimen was examined for the GDH antigen using the VIDAS GDH assay, and, if GDH was positive, for toxins using the VIDAS CDAB assay in a separate cassette. The assays involve a two-step sandwich immunoassay using the enzyme-linked fluorescence immunoassay technique. Aliquots of stool were added to a dedicated diluent. After centrifugation, the supernatant was used for testing, and the results were interpreted using an optical density (OD) cutoff at 450/630 nm on a spectrophotometer according to the manufacturer's instructions.

Samples from the VIDAS GDH assay were defined as negative with an OD <0.10 and positive with an OD ≥0.10. For the VIDAS CDAB, a fluorescence intensity of <0.13 indicated a negative test, ≥0.13 to <0.37 an equivocal test, and ≥0.37 a positive test. An equivocal result was considered negative for the performance calculations in this study.

All stool samples were processed for GDH; if they were positive, they were tested for toxins A and B. If GDH was positive, toxins A and B were negative, indicating colonization. If GDH was negative, Toxin A and B were not performed.

RESULTS

A total of 124 healthcare workers consented to participate in the study. Of these, 100 submitted stool samples and were included in the final laboratory analysis, yielding an 80.6% response rate. The demographic distribution of the study population showed a predominance of female participants, who accounted for nearly three-fourths of the cohort, while males constituted approximately one-fourth. This distribution reflects the workforce pattern commonly observed in healthcare institutions, particularly among nursing professionals.

Among the study population, 73% were female, and 27% were male. Nursing staff constituted the majority of participants (76%), while the remaining 24% were other healthcare workers. A history of contact with patients with gastrointestinal tract (GIT) infections was reported by 10% of participants. GDH antigen positivity for C. difficile was detected in 4% of samples, whereas none tested positive on the toxin A/B assay. The results are presented in Table 1.

Table 1: Tabular representation of the results

CHARACTERISTICS	PERCENTAGE
FEMALE POPULATION	73
MALE POPULATION	27
NURSING STAFF	76
OTHER HEALTH CARE WORKERS	24
CONTACT WITH GIT PATIENTS	10

TESTED (+) FOR GDH ANTIGEN	4
TESTED (+) FOR A/B TOXIN	0

The occupational profile of the participants showed that the majority of the study population was nursing staff, representing nearly three-fourths of all participants. The remaining participants included healthcare workers from various hospital departments involved in direct or indirect patient care and hospital support services. The study specifically included personnel working in high-risk patient-contact environments, such as Intensive Care Units (ICUs), inpatient wards, and food supply units, where exposure to infectious agents is more likely due to frequent interaction with hospitalized patients and contaminated environments.

Among the 100 participants who provided stool samples, 10 healthcare workers reported a history of direct contact with patients suffering from chronic diarrhoea and other gastrointestinal infections during their routine clinical duties. These participants were considered to have an increased risk of occupational exposure to enteric pathogens, including C. difficile.

Laboratory screening of all stool samples was performed using the Glutamate Dehydrogenase (GDH) assay as the initial method for detecting C. difficile. Of the 100 samples analyzed, four samples (4%) tested positive for GDH antigen. However, subsequent testing for C. difficile Toxins A and B showed negative results in all four GDH-positive samples. The remaining 96 samples were negative for the GDH assay, indicating the absence of detectable C. difficile antigen.

GDH-positive but toxin-negative findings may indicate asymptomatic colonization or the presence of non-toxigenic C. difficile strains among healthcare workers rather than active toxigenic infection. No participant showed laboratory evidence of toxin-producing C. difficile infection during the study period. These findings indicate a low prevalence of toxigenic C. difficile carriage among healthcare workers in this study setting, despite occupational exposure to high-risk clinical environments.

DISCUSSION

Of the 100 tested samples, 4 tested positive for the GDH (Glutamate Dehydrogenase) Antigen. After testing for the GDH Antigen, those samples were tested again for the A/B Toxin for the C. difficile infection. The majority, i.e., three-fourths of the population, were nursing staff. The four positive samples for the GDH Antigen were from the nursing population (75%) and the sanitation workers (25%). The females were positive at 75%, i.e., three of the four samples that tested positive were from females.

Surveillance data from 2021, conducted by the CDC for the HAI tracking of CDI in the USA, stated that the overall incidence rate of CDI in 2021 was 110.2 cases per 100,000 persons, with a slightly higher incidence of community-associated cases (55.9 cases per 100,000 persons) compared with healthcare-associated cases (54.3 cases per 100,000 persons) in the country. The incidence rate of CDI increased with age and was higher in women than in men and higher in White persons than in persons of other races among the US population. CDI-related complications, such as toxic megacolon and ileus, were rare.

Existing research on healthcare worker colonization with C. difficile has yielded conflicting results. Studies published in 1998 and 2012 did not detect C. difficile stool colonization among 55 and 112 healthcare workers, respectively. In contrast, C. difficile carriage was detected in 4 of 30 HCWs (13%) by van Nood, and in 4.3% of hospital staff by Kato. These data indicate that colonization among HCWs typically occurs at a similar frequency to that among healthy adults and that a high level of occupational exposure would be required to result in colonization or infection among HCWs. Arfons and others suggest that C. difficile may be an underappreciated occupational risk for healthcare workers. Many healthcare workers are immunocompromised or have chronic medical conditions, and antibiotic treatment in the preceding year has been reported by nearly half of healthcare workers.[19,20,21]

The Meta-analysis study done on the prevalence of C. difficile contamination in the healthcare environment and instruments, using the main databases like PubMed, Google Scholar, etc., has found India to have the highest prevalence of CDI, with 51.1%, and the USA to have the lowest prevalence of CDI in a hospital setting.

More than 70% of all CDI cases globally are hospital-associated. Many studies have reported that environmental contamination with CD could increase the risk of CDI in hospitalized patients receiving antibiotics. Proximity to high-touch, contaminated surfaces and devices is positively correlated with CDI. Therefore, implementing precise surveillance and screening of high-risk, high-touch surfaces in hospital wards could help control HAI, especially CDI.[17]

In the present study, healthcare workers, including nurses, caretakers, and sanitation workers, were recruited. These categories of healthcare workers were chosen because of their direct patient contact. Our volunteers in this point prevalence study were predominantly female nurses, as was found in Hell's study. Of all HCW groups, nursing staff are likely to have the closest contact with patients and be at the greatest risk of exposure to stool.

The actual difficulty of this study was obtaining stool samples from participants, as stool collection is a taboo topic for the public. The number of stool samples collected is also lower for the same reason. Testing the stool sample after proper preservation and decontamination of the specimen to identify the specific toxin/antigen was quite tedious. Due to the

difficulty of screening tests for C. difficile infection in asymptomatic carriers, these tests are not performed as part of routine screening. The emergence of CDI as a hospital-acquired infection is a major evolving threat to the healthcare system and also lacks the required awareness among physicians and healthcare workers. The development of easier, faster methods of screening for CDI would have increased the number of screening tests performed in a tertiary care setting. Recent studies have shown that, even though the asymptomatic carriers don't suffer directly from CDI, they serve as a potential factor in the chain of infection, turning HAI into a Community-acquired disease. They also laid the groundwork for the emergence of antibiotic-resistant, more virulent strains of C. difficile.[20,21]

The present study, conducted on asymptomatic health care workers, proved significant. It might be due to the small sample size and the inclusion criteria of the asymptomatic population. The nursing staff who had close contact with the patients tested positive, mostly due to greater exposure to the agent.

CONCLUSION

The study was conducted to determine the number of asymptomatic C. difficile carriers in the district hospital setting who could be healthy carriers transmitting C. difficile infection (CDI) to patients as a hospital-acquired infection. CDI is a serious healthcare-associated infection (HAI) increasingly reported from hospitals across India. However, there is a paucity of data on the incidence of CDI and the impact of control measures on CDI in India. All hospitals need to establish policies for the surveillance, testing, treatment, and prevention of CDI, based on recent international guidelines and local infrastructure and logistics. The experiment was conducted among the healthcare workers of the district hospital. Stool samples were collected after obtaining consent. The samples were tested for the GDH (glutamate dehydrogenase) antigen, and those that tested positive were further tested with an immunoassay to detect Toxin A/B, confirming colonization and ruling out infection. Those who tested positive for GDH and negative for Toxin A and B were considered C. difficile suspected carriers and potential risk factors for HAI among patients. The current study showed that 4% of the 100 stool samples from healthcare workers tested positive for C. difficile. Thus, this experiment indicates a higher likelihood of CDI as an emerging HAI due to reduced screening of potential carriers and increased exposure to the organism among health care workers.

CDI poses a growing public health concern in India. The present study, conducted among health care workers at the district hospital in Coimbatore, Tamil Nadu, found that 4% tested positive for C. difficile infection. Most of the study population were health care workers who had close contact with patients, thus signifying the chain of infection through health care workers as healthy carriers of hospital-acquired CDI. Further studies are required to determine the actual extent and incidence of CDI among health care workers across various hospital settings, including the incidence and prevalence of CDI across hospitals in the region. The awareness among the healthcare workers about the emergence of CDI is not sufficient. Thus, further detailed study of the risk of CDI as an emerging infection globally must be conducted among healthcare workers, reporting potential exposure and spread of CDI through them as carriers.

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