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Association of healthcare exposure with acquisition of different *Clostridium difficile* strain types in patients with recurrent infection or colonization after clinical resolution of initial infection

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SUMMARY

Background: Following the resolution of an episode of *Clostridium difficile* infection (CDI), the factors associated with acquisition of different *C. difficile* strain types in patients with recurrent infection or persistent colonization have not been evaluated.
Aim: To explore factors with potential correlation with acquisition of different *C. difficile* strain types in patients clinically cured of CDI through long-term follow-up across the continuum of care.
Methods: Polymerase chain reaction ribotyping was performed on *C. difficile* isolates recovered at baseline and follow-up (days 19–38) from stool samples of patients successfully treated for CDI, and those who had recurrence and/or colonization following symptom resolution. Chart review was conducted to determine factors associated with acquisition of a different *C. difficile* ribotype.
Findings: Of 25 patients initially cured of CDI, five had a recurrence and eight were colonized at follow-up. Patients did not differ with regard to age, sex, and whether the initial infection was with the BI/NAP1/027 strain. Ribotyping revealed that two out of five patients had recurrence attributed to a different strain type. Three of the colonized patients demonstrated strain switching compared with five patients who carried the same baseline strain. All patients (both infected and colonized) with different *C. difficile* ribotypes were exposed to the healthcare system. Exposure to antibiotics and proton pump inhibitors were not related to strain switching.

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Conclusion: Exposure to healthcare, but not to antibiotics or proton pump inhibitors, was consistently associated with recurrence or colonization with a different *C. difficile* ribotype.

Introduction

Approximately 15–35% of patients with an initial *Clostridium difficile* infection (CDI) are at risk of recurrence of infection within two months.[1] Overall, the high incidence of CDI poses a significant financial burden with estimated annual treatment costs of more than $3 billion in the USA alone.[2]

Clostridium difficile is associated with a wide range of clinical manifestations, from asymptomatic colonization, moderate to severe diarrhoea, and, at its most severe form, death. Although CDI itself is clearly important, colonization with *C. difficile* should not be understated. *Clostridium difficile* colonization increases the risk of CDI up to six times compared to non-colonized patients.[3] Additionally, the rate of *C. difficile* colonization is estimated to be more than 8% among hospitalized patients, and hospitalized patients are 63% more likely to be colonized with *C. difficile* when compared with non-hospitalized controls.[3,4]

Molecular typing of *C. difficile* has greatly advanced over the last decade with polymerase chain reaction (PCR) ribotyping being the most widely used method serving this purpose.[5] One of the applications of this technology is the identification of *C. difficile* strains isolated from patients with CDI recurrence in order to assess their similarity to the strains acquired at the initial episode (index strain).[6,7] Factors associated with the acquisition of a *C. difficile* strain that is similar or different from the index strains have not yet been well described. Therefore, the objective of this study was to explore these factors and their potential correlation with this outcome through long-term follow-up of CDI patients across a continuum of care.

Methods

Patients and samples

A prospective study of patients with CDI was conducted at Hartford Hospital, Hartford, CT, USA. Stool samples from enrolled patients were collected at baseline and during a follow-up period (19–38 days post therapy initiation). These samples were sent to Washington University, St Louis, MO, USA for PCR ribotyping of isolated *C. difficile* strains.[8] Only patients experiencing a primary episode of CDI were eligible to be enrolled in this study where they were randomized to receive either vancomycin 125 mg every 6 h or fidaxomicin 200 mg every 12 h. The study protocol was approved by the Hartford Hospital Institutional Review Board and all patients provided written informed consent prior to study enrolment.

PCR ribotyping

PCR ribotyping was performed using a laboratory-developed semi-automated PCR-ribotyping platform as previously described.[9] Briefly, DNA was extracted from the isolates using the MO BIO Bacteraemia DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA), and ~100 ng of DNA was used per reaction. PCR was performed in a 25 μL PCR mixture, using PuReTaq Ready-To-Go™ PCR Beads (GE Healthcare, Little Chalfont, UK) and PCR ribotyping primers as previously described.[9] Results were resolved using DiversiLab DNA chips (bioMérieux, Durham, NC, USA) and the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). DiversiLab Bacterial Barcodes software program was used to analyse the resultant banding patterns. The data were then organized into a dendrogram, and a similarity index (SI) for each pair was calculated. SI ≥95% was required to call two strains identical.

Data extraction

Chart review was conducted in order to identify factors potentially associated with the acquisition of different *C. difficile* ribotypes in patients who were clinically cured of their initial CDI episode. Clinical cure was defined as complete resolution of CDI clinical signs and symptoms, including normalization of stool consistency and reduction of stool frequency to less than three unformed stools per day, without the need for further treatment. Factors that were investigated included age, sex, initial infection with BI/NAP1/027 strain (given its known association with severe disease), exposure to the healthcare system (including hospitalization, chronic dialysis, admission to the observation unit of the emergency department, and residence in a long-term care facility), exposure to antibiotic therapy, exposure to proton pump inhibitors (PPIs), and initial CDI therapy (fidaxomicin or vancomycin). PPIs are known for their association with a high risk of hospital-acquired CDI, as well as CDI recurrence.[10,11] Exposure to these factors was evaluated post clinical cure of CDI and before stool sample collection at follow-up.

Definitions

In order to distinguish between patients who developed recurrence due to the same or different *C. difficile* strain as compared to the index strain, the following definitions were utilized as previously established.[6,7] CDI relapse was defined as recurrence of CDI due to the same index strain; whereas CDI reinfection was defined as development of CDI symptoms due to the acquisition of a strain that was different from that of the baseline. Colonized patients were those who carried *C. difficile* without exhibiting CDI symptoms (i.e. asymptomatic carriers). Patients who were colonized with *C. difficile* were contacted via telephone 10–14 days after the collection of the follow-up sample in order to assess whether or not they developed symptomatic *C. difficile* infection.