A Clinical Epidemiological Study of Clostridium difficile Infection at the University of Chicago Hospital
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ABSTRACT

Clostridium difficile is a Gram-positive, obligately anaerobic, and spore-forming bacterium. C. difficile is found commonly in soil and water but can cause disease in humans and warm-blooded animals. C. difficile infection (CDI) typically results from disruption of the intestinal microbiome by broad-spectrum antibiotic use. CDI has become the most common healthcare-associated infection in the United States, and costs $4.8 billion each year to emergency medical facilities alone. Recent increases in the prevalence and severity of CDI have created a need to understand the epidemiology of different strains (genotypes) of C. difficile to determine what healthcare professionals and infection control personnel can do to more effectively mitigate this disease. In this project, 180 stool samples from the University of Chicago Medical Center (UCMC) were cultured and genotyped by PCR ribotyping to establish a baseline molecular epidemiology for this facility. This was done using both a multiplex PCR gel electrophoresis and fluorescent PCR ribotyping techniques. The most abundant strains were F014-020, F027, and FP311 respectively. Our results suggest that there was an outbreak of ribotype F014-020 at UCMC, although more analysis needs to be done within each given ribotype to establish how genetically related they are and whether there is significant evidence of patient-to-patient transmission.

INTRODUCTION

Clostridium difficile gets its name from both Latin and Greek origins; “kloster,” which means spore in Greek because of its bacillus morphology (Fig. 1), and “difficile”, or difficult or obstinate in Latin, because it is difficult to culture; difficult to kill in its spore form, and it is resistant to genetic manipulation.

METHODS

Isolation and Cultivation of C. difficile

An ethanol shock was performed on each stool sample to kill off other bacteria and select for ethanol-resistant C. difficile spores. For this, an aliquot of stool was added to an equal volume of 100% ethanol and incubated for 1 hour. This slurry was then streaked for isolation onto pre-reduced (anaerobic) taurocholate cysteine clostridial (TCCFA) plates and incubated anaerobically at 37°C for 24 hours. Single colonies were selected and sub-cultured in BHIS. A 1:10 dilution of this overnight culture was used as a template DNA for all PCR reactions.

Fluorescent PCR Ribotyping

This method was developed by Dr. Seth Walk, as a cost effective, accurate, partial genome analysis (1). The method detects differences in length between the 16S rRNA and 23S rRNA encoding genes. There are up to 14 different rRNA operons in the C. difficile genome, and so a total of 14 different fragments could be generated in a single isolate. PCR-generated fragments are accurately sized using capillary electrophoresis to generate peaks on a chromatogram (Fig. 4). These distribution of peaks are then analyzed against a database at the Walk Lab (thewalklab.com/tools/) that matches them with known ribotypes with the same peaks.

Toxin Gene PCR

A multiplex PCR developed by Persson et al (2) was used to detect C. difficile toxin genes A (cdtA) and B (cdtB) as well as the binary toxin genes, cdtA and cdtB (Fig. 5). An internal control gene targeting the 16S rRNA encoding gene was also detected.

RESULTS

The abundance of ribotype F014-020 (Fig. 8) was somewhat surprising, and is consistent with the possibility of patient-to-patient spread as part of one or more recent outbreaks at UCMC. In contrast, an abundance of F027 was expected. This ribotype was common, at least in some hospitals, from 1962-1986, but then seemingly disappeared by 1987 without any radical change in the rate of CDI (3). Beginning in the early 1990’s through the first decade of the 21st century, a pandemic occurred that was driven by epidemic clones of F027, where patients were more likely to be admitted to an intensive care unit, require an emergency surgery (e.g. colectomy), or die from the infection (3). Now, this ribotype seems to be decreasing in prevalence, whereas other ribotypes appear to be on the rise (3). Our results represent only a snapshot of the ribotype diversity at UCMC, and characterization of another set of stool samples is necessary to understand whether ribotypes, such as F027, are increasing or decreasing.

CONCLUSIONS

PCR ribotype F014-020 was the most abundant C. difficile genotype at UCMC, comprising over 25% of all isolates. Further analysis of these isolates is needed to confirm that an outbreak has occurred or is still actively taking place. Our results support previous reports that F027 is no longer the most abundant ribotype in hospitals. Increased surveillance of C. difficile genotypes will ensure that temporal dynamics are captured, in order to stay ahead of other C. difficile pandemic clones.

FUTURE WORK

For outbreak investigations, a more discriminant PCR-based method, called multilocus variable-number tandem repeat analysis (MLVA), can be used to characterize these isolates. This technique resolves rapidly evolving changes that occur at direct nucleotide repeat regions of the C. difficile genome after only a few (~10) cycles of replication (4). This technique is excellent for discriminating between isolates of the same ribotype involved in patient-to-patient spread or possibly healthcare worker-to-patient spread. An additional collection of isolates from UCMC would be necessary to understand temporal ribotype dynamics.

DISCUSSION

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CITATIONS


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