Poster #2230

Are different ribotypes of *Clostridium difficile* present simultaneously in a patient with a *Clostridium difficile* infection?

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Abstract

Clostridium difficile is the most common pathogen causing nosocomial infectious diarrhoea. Toxin A and toxin B of *C. difficile* are responsible for infectious diarrhoea. *C. difficile* is commonly found in our environment and the major contributing factor to its spread is *C. difficile* spores. The aim of our study was to investigate if different *C. difficile* isolates are present in a single sample in respect to ribotype and toxin production. The majority of the samples showed no difference in either ribotype or toxin production. However, we have been able to show one sample with two different ribotypes and different pattern of toxin production.

Introduction

Infection with toxin-producing *Clostridium difficile* strains is a common cause of diarrhea and colitis. *C. difficile* infection (CDI) has increased in frequency and severity in North America and Europe over the last decade largely due to the emergence of the epidemic PCR ribotype 027 strain. PCR ribotyping is based on a comparison of patterns of PCR products from the 16S-23S rRNA intergenic spacer region. Isolates are considered to be of a new PCR ribotype if the pattern is at least one band different from previously described patterns. The aim of this study was to investigate whether CDI is caused by multiple toxigenic strains or by a single strain.

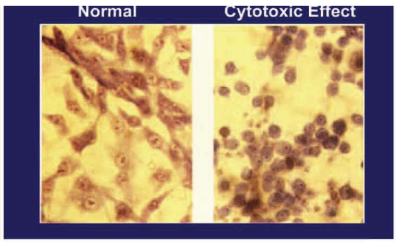

Figure 1. CCNA assay of clinical *C. difficile* isolates

Methods

Colonies of *C. difficile* isolates were collected from each stool sample of 28 *C. difficile* positive patients. The stool samples were cultured on TCCFA agar plates and five different colonies were subcultured on blood agar plates in anaerobic conditions for 48 hours. In total 140 isolates of *C. difficile* were included in the study. Toxin B was detected by cell culture neutralisation assay (CCNA) (Figure 1). In addition, all isolates were tested with the Cepheid Xpert™ realtime PCR (Figure 2). PCR ribotyping was used to analyse the different isolates of *C. difficile*. Ribotyping PCR products were separated on 5% polyacrylamide gel electrophoresis (Figure 3). The gels were scanned and analyzed by Bionumerics software version 6.5. PCR ribotyping patterns were compared to a database including *C. difficile* reference strains.

Figure 2. Cepheid Xpert™ realtime PCR assay instrument

Results

Overall, 12 different ribotypes were found in the 28 samples. The most common ribotypes were 001, 002, 014 and 078, four samples each. All isolates from each individual sample showed the same ribotype except in one sample where 4 colonies were of the same ribotype and one colony was different. The colony that differed was shown to be non-toxigenic by both the CCNA assay and Cepheid Xpert™ realtime PCR assay.

Conclusion

The results from the ribotyping of the 28 stool samples indicate that CDI is usually caused by one particular C. difficile ribotype. In this study it is shown that CDI is not caused by multiple toxigenic strains.

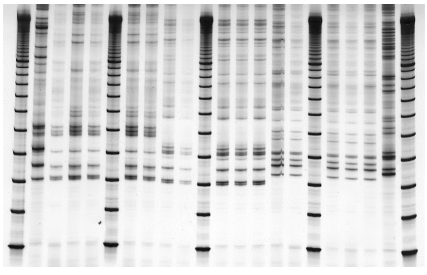

Figure 3. PCR-ribotyping of clinical *C. difficile* isolates

References

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