P2230

Abstract (poster session)

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

M. Lewandowski, M. Rashid, C.E. Nord, A. Weintraub* (Huddinge, SE)

Objectives: Infection with toxin-producing Clostridium difficile strains is a common cause of diarrhea and colitis. Clostridium 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. 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). In addition, all isolates were tested with the Cepheid Xpert™ realtime PCR. PCR ribotyping was used to analyse the different isolates of C. difficile. Ribotyping PCR products were separated on 5% polyacrylamide gels by electrophoresis. 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. 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 the CCNA 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.