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Abstract (poster session)

Characterisation of Clostridium difficile strains isolated in Italy from 2007 to 2011 and comparative analysis of the predominant polymerase chain reaction (PCR)-ribotypes

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Objectives. To characterize the Italian C. difficile strains, isolated between 2007 and 2011, and to compare the predominant PCR-ribotypes. Methods. 132 strains, sent from different Italian hospitals to ISS for characterization, were typed using capillary-gel-electrophoresis based PCR-ribotyping and investigated for antibiotic susceptibility to moxifloxacin (MXF), erythromycin (ERY), clindamycin (CLI) and rifampin (RIF) by Etest. Thirty two selected strains were further characterized for antibiotics resistance mechanisms by amplification and sequencing of the target genes, for the slpA gene by PCR-mapping and sequencing and for sporulation through cultures in BHIS broth in anaerobic conditions at 37°C for 5 days. Moreover, “in vitro” adhesion proprieties were investigated on Caco-2 cells at 15 days (post confluent monolayers) and competitive assays were carried out culturing the strains in BHI broth for 24 hours, mixing the cultures in a 1:1 ratio, transferring a dilution of the mixture into fresh broth every 24 hours for three cycles and counting the number of viable cells by plating on selective medium at the end of every cycle. Results. Among the 132 strains analyzed, the predominant PCR-ribotypes were 018 (69%) and 078/126 (14%), followed by 15 others types. The ratio between 018 and 078/126 percentage increased throughout the period of study. 73% of the strains were multi-resistant and the majority of them (88%) were strains 018. In resistant selected strains, 018 or 078/126, an ermB was detected only in strains highly resistant to both ERY and CLI. Resistance to MXF and RIF was associated to amino acid substitutions in GyrA (Thr82 to Ile) and in RpoB (Ser498 to Thr and/or Arg505 to Lys), respectively. As far as the surface layer (S-layer) is concerned, two new SlpA variants, showing high identity with those of strains 078/126, were found in strains 018. Sporulation was strain-dependent but more abundant for strains 018 after 48h. A significantly higher adhesion to CaCo-2 cells was observed for strains 018. Furthermore, growth competition assays indicated that strains 018 rapidly overcome strains 078/126 with a decrease in fitness for the last. Conclusion. PCR-ribotype 018 shows peculiar characteristics that allow it to overcome PCR-ribotype 078/126 in “in vitro” assays. These characteristics may have played a role in the rapid spread and persistence of PCR-ribotype 018 in our Country .