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Abstract (poster session)

Characterisation of *Clostridium difficile* 018: an epidemic PCR-ribotype recently emerged in Italy

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Objectives. To characterize *C. difficile* PCR-ribotype 018, a recent epidemic type cause of numerous outbreaks and sporadic cases in Italy, in comparison with other PCR-ribotypes predominant in the past. **Methods.** 242 toxinogenic clinical strains, isolated from 1985 to 2011 and collected by the Istituto Superiore di Sanità, were typed by the PCR-ribotyping method. Two different strains PCR-ribotype 018, one strain 126 and one strain 012, were further investigated as representative of different hospital outbreaks occurred in different time periods. The MICs for erythromycin (ERY), clindamycin (CM) and moxifloxacin (MX) were evaluated by the Etest. *ermB* genes were detected by PCR assay. TcdC, the toxin negative regulator, and SlpA, the S-layer proteins precursor, were characterized by sequencing. In vitro adhesion was performed on Caco-2 monolayers at 3 and 15 days after seeding and adherent bacteria were counted on Blood agar plates after 48h of incubation. Different dilutions of filtered supernatants from overnight cultures of the strains were used for cytotoxicity assay on confluent Caco-2 cell monolayers. **Results.** Results indicated PCR-ribotype 018 as the predominant type in Italy since 2006, while PCR-ribotypes 126 and 012 were those most frequently isolated in the previous years. All the representative strains analysed were resistant to ERY, whereas one of the two strains 018 and the strain 012 were also resistant to CM and *ermB*-positive. All strains were resistant to MX out of strain 012. Sequence analysis identified the SlpA of PCR-ribotype 018 as a new variant, with an identity of 89% and 57% compared to those found in strains 126 and 012, respectively. In all assays performed, strains 018 showed a number of adherent bacteria per cell significantly higher compared to the other strains. A mutated TcdC was observed only in strain 126. As far as cytotoxicity is concerned, strains 018 showed an earlier activity visible as cell damage at 6 hours. **Conclusion.** PCR-ribotype 018 is a new epidemic *C. difficile* type showing peculiar characteristics, such as a new SlpA variant, an increased adhesiveness on Caco-2 cell monolayers and an earlier cytotoxic activity, which may play a role in the enhancement of virulence and in facilitating the spread of these strains.