

Abstract

Currently, *Clostridium difficile* is a leading nosocomial pathogen due to the spread of epidemic strains. Molecular typing of clinical isolates is an important part of *C. difficile* occurrence and spread control in hospitals as well as in the community.

A total of 2201 clinical *C. difficile* isolates from 32 hospitals cultured between 2013-2015 were characterized by PCR ribotyping and toxin gene multiplex PCR. A total of 166 different ribotyping profiles were identified, of which 53 ribotyping profiles were represented by at least two isolates for each profile. The most frequently found ribotypes were 176 (n=588, 26.7%) and 001 (n=456, 20.7%) followed by 014 (n=176, 8%), 012 (n=127, 5.8%), 017 (n=85, 3.9%) and 020 (n=68, 3.1%). Out of 2201 isolates, 2024 (92%) isolates were toxigenic and carried genes for toxin A and B, and of these, 677 (33.5%) also carried genes for binary toxin. The remaining 177 (8%) isolates were non-toxigenic.

Subtyping of *C. difficile* isolates using a multilocus variable-number tandem repeats analysis (MLVA), that compared the sum of tandem repeats differences, was performed in *C. difficile* isolates of ribotype 176 (n=225, 17 hospitals) and in *C. difficile* isolates of ribotype 001 (n=184, 14 hospitals) cultured in 2014. The clonal relatedness in *C. difficile* isolates belonging to the same ribotype was found in 76.6% of ribotype 001 isolates forming 14 clonal complexes and in 84.5% of ribotype 176 isolates forming 27 clonal complexes. MLVA was also used in three retrospective local department epidemiologic investigations (78 isolates of ribotype 176 from 2013 and 11 isolates of ribotype 001 from 2014) with similar results. That clonal relatedness of *C. difficile* isolates of ribotypes 001 or 176 was observed within and between hospitals confirms a higher potential of these ribotypes to spread in a hospital environment.

The phylogenetic relationship of selected isolates belonging to different ribotyping profiles was investigated using multilocus sequence typing (MLST). The selected 53 *C. difficile* isolates revealed 40 different sequence types. Ribotypes with the same sequence type also had a similar ribotyping profile and carried the same toxin genes which suggest a phylogenetic relatedness.

The significant representation of the two ribotypes revealed in a representative number of hospitals, could be regarded as an epidemiologically important situation. The data obtained can be used for further the monitoring and assessment of the dynamics of distribution and representation of individual *C. difficile* ribotypes in the country.

