

ASSOCIATION BETWEEN MOTILITY, RESTRICTION FRAGMENTS LENGTH POLYMORPHISM TYPE OF FLAGELLAR GENE *fliC* AND RIBOTYPES OF *CLOSTRIDIODES DIFFICILE*

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Background

Clostridioides difficile is one of the most important hospital pathogens of the 21st century. The reason for the increase in the number of *C. difficile* infections, among others, was the appearance of „hypervirulent” strains as PCR-ribotype 027 (RT027). The aim of this study was to investigate the motility of hypervirulent strains in comparison with other PCR-ribotypes (RTs) and to study the relationship with restriction patterns of *fliC* genes (RFLP). The clonal relatedness of strains was also determined.

Material and methods

Motility and presence of the flagellin gene *fliC* was studied in 82 *C. difficile* isolates, derived from non-duplicated patients hospitalized in different regions in Poland, belonging to the RTs: 027 (n=41), 176 (n=17), 023 (n=8) 017 (n=6) and 046 (n=10). Two reference strains (motile *C. difficile* 630, RT012, and non-motile *C. difficile* M120, RT078) were used as controls for motility assay. The variability of *fliC* gene was further studied by PCR-restriction fragment length polymorphism (RFLP) analysis. The Multiple Locus Variable-number Tandem Repeat Analysis (MLVA) was used to study the genetic relatedness of *C. difficile* strains belonging to the same RT. A Minimum Spanning Tree (MST) was created by using the Manhattan coefficient.

Results

In the motility assay, RT023 strains showed the highest motility ($p < 0.01$). Strains of RTs 017 and 046 were less motile than „hypervirulent” strains (RTs 027, 176, and 023) or were non-motile (Fig 1). The *fliC* gene was present in all clinical strains in both motile and non-motile. Five PCR-restriction fragment length polymorphism (RFLP) groups were identified; three previously published by Tasteyre et al., 2000 (I, II, VII) and two new RFLP groups X and XI. Among them, group VII corresponded to RT027 and RT176. One strain RT027 belonged to new RFLP group X. All RT023 strains belonged to new group XI. Among RT017 two RFLP groups were detected (I and II), the majority belonged to II group. Only selected RFLP of *fliC* genes (RT027 and RT176) as showed in Fig. 2 and Fig. 3. All strains of RT046 belonged to type I. MLVA showed genetic non-relatedness between majority strains in the study.

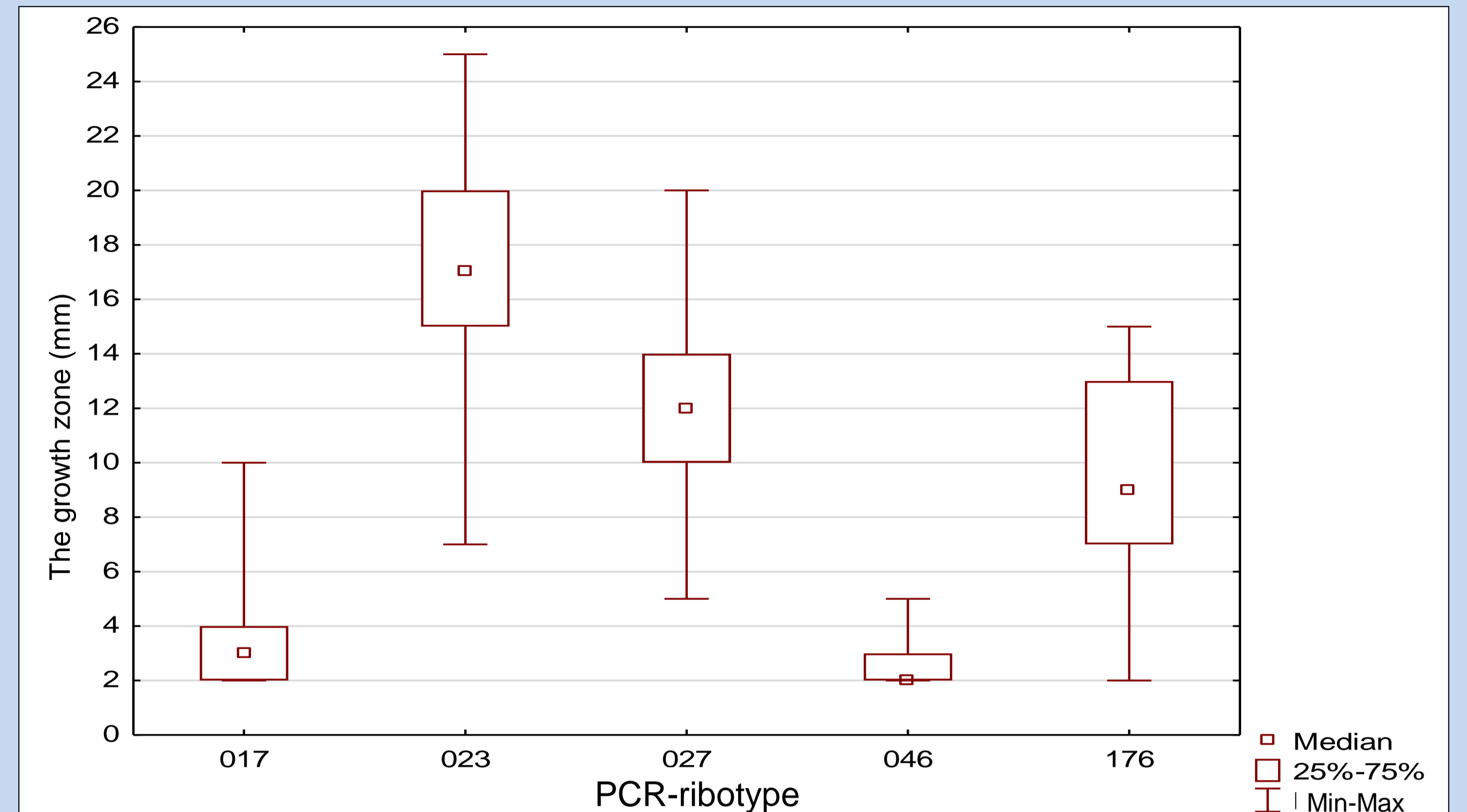


Fig. 1. Average colony diameter illustrating the motility of clinical strains belonging to different PCR-ribotypes (RTs).

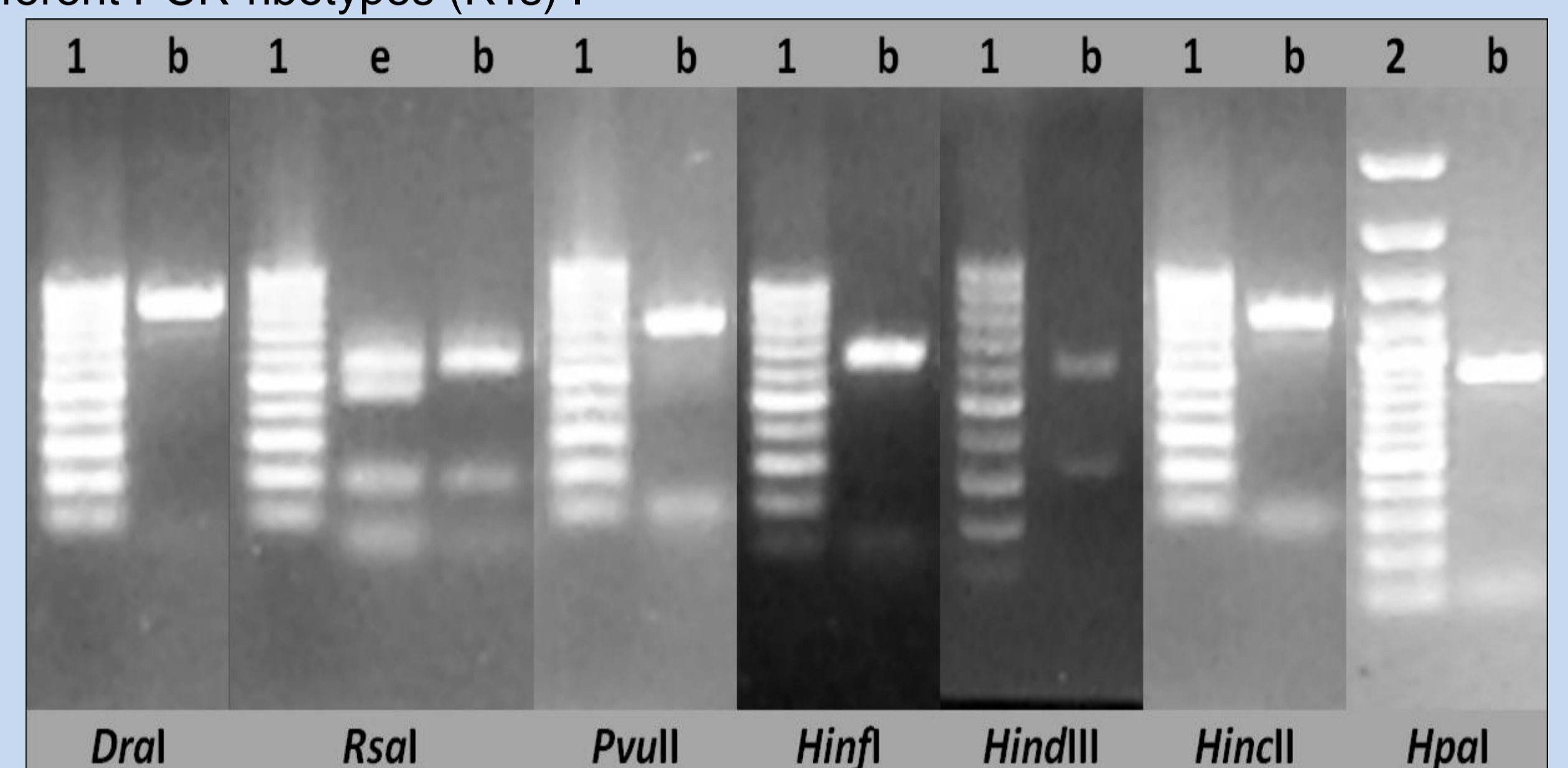


Fig. 2. Results of restriction endonuclease cleavage of the fragment of the *fliC* gene, amplified by PCR with DNA of *C. difficile* RT027 strains. Legend: 1, 2 - M1 and M2 patterns of DNA fragment sizes; b, e - RFLP patterns

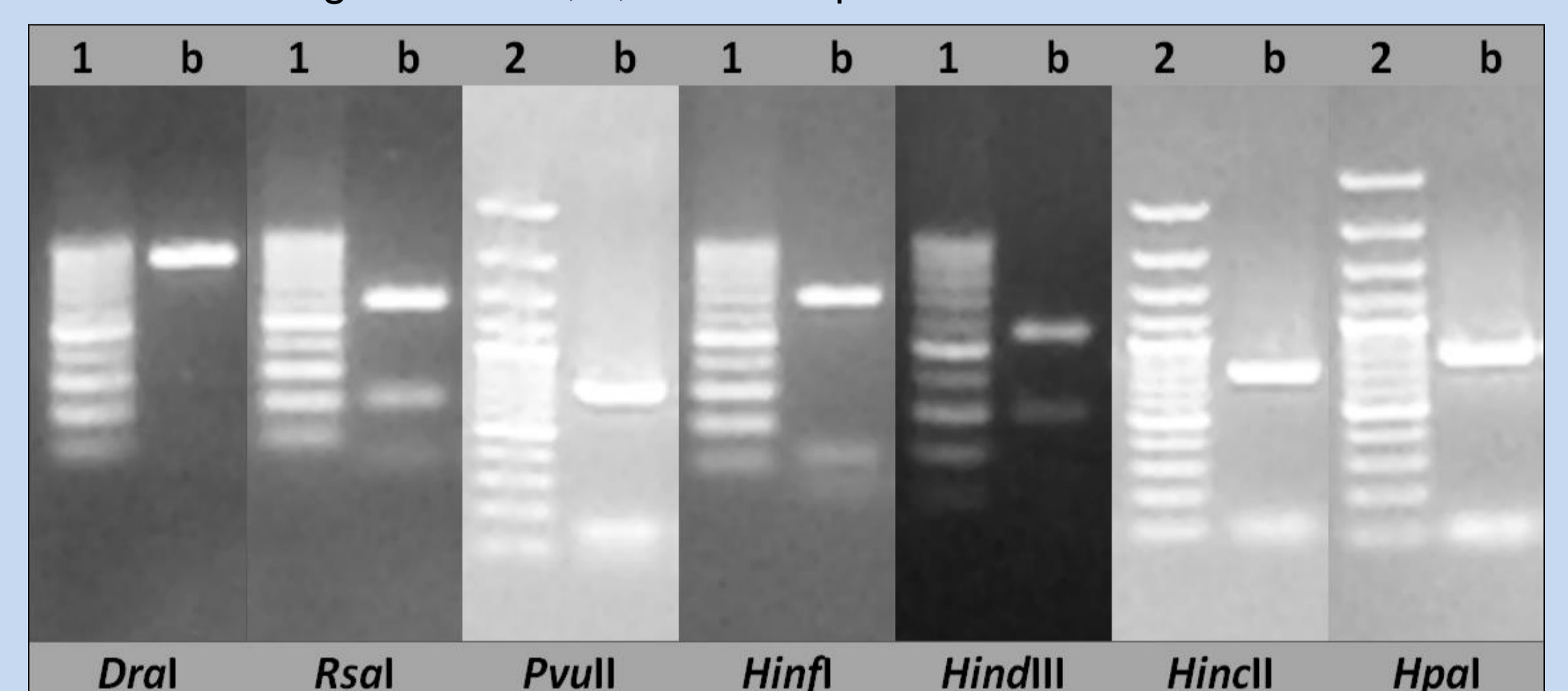


Fig. 3. Results of restriction endonuclease cleavage of the fragment of the *fliC* gene, amplified by PCR with DNA of *C. difficile* RT176 strains. Legend: 1, 2 - M1 and M2 patterns of DNA fragment sizes; b - RFLP pattern

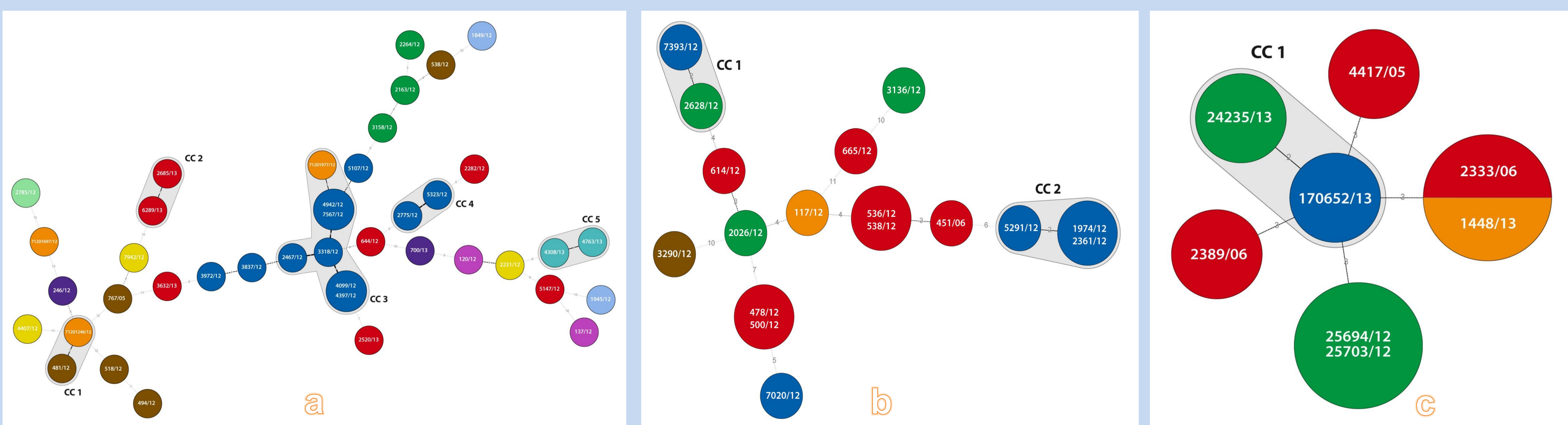


Fig. 4. Minimum spanning tree (MST) of *C. difficile* strains a) RT027; b) RT176, c) RT023. Clonal complex (CC) was defined as the sum of tandem repeat differences (STRD) ≤ 2 (grey). The numbers in circles represent the DNA number of *C. difficile* isolates. The numbers on the lines represent the sum of tandem repeat differences (STRD) between isolates. If more than one number is present in one circle, it represents isolates with STRD=0. Each hospital is represented in different colour.

Conclusions

„Presumably” hypervirulent RTs 023 and hypervirulent 027 and 176 showed to be more motile compared to RTs 017 and 046 and controls. Interestingly, two RFLP patterns in one RT were observed. Further studies on a larger number of isolates and ribotypes are needed to confirm our results.

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