GENOTIPIC AND PHENOTIPIC CHARACTERIZATION OF THREE NOVELS CLADE 2 *Clostridium difficile* BRAZILAN ISOLATES

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INTRODUCTION

The epidemiology of CDI is highly dynamic and new strains continue to emerge worldwide. The increased incidence and severity of disease at a global scale has been linked to the emergence and spread of epidemic strains belonging to MLST clade 2 and 5, mostly ribotypes (RT) 027 and 078. In contrast with most countries, these classic epidemic strains seems rare in Brazilian hospitals. On the other hand, isolation of novel strains from clade 2 has been reported in some hospitals. Thus, to better understand the hypervirulent potential of some novel strains, the present study aimed to characterize phenotypically and genotypically three new strains and compare them to *C. difficile* RT027 (strain CD196).

MATERIAL AND METHODS

The strains were previously described by Diniz et al. (2019). They were classified into new ribotypes/sequence types an identified as RT 883/ ST 461 (HC27), RT 884/ ST 462 (HC58) and RT 885/ ST 463 (HC76). First, these strains were submitted to WGS and then characterized phenotypically by: A/B toxin production, sporulation rate, swimming motility in culture medium and lethality in hamster model.

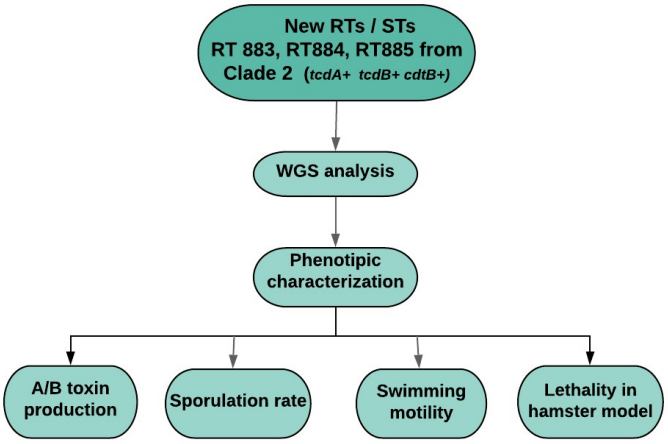

Figure 1: Experimental design of genotypic and phenotypic characterization from RT 883, RT884 and RT885.

RESULTS AND DISCUSSION

The WGS analysis revealed a high similarity among these strains and suggested that they emerged from a common ancestor related to Clade 2 strains. On figure 2 the cgMLST analysis shows that RT883,884 and 885 are very similar to each other and they are related to others clade 2 reference strains (Figure 2).

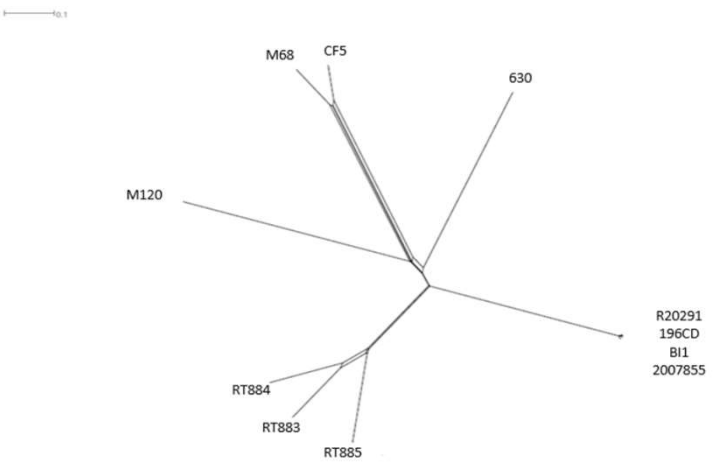

Figure 2: NeighbourNet diagram showing the population structurre of RT883,884, 885 and reference strains from clade 1 (630), 2 (R20291, 196CD,BI1,2007855), 4 (M68 and CF5) and 5 (M120).

New *tcdB* and *tcdC* alleles were identified and deposited on PubMLST database as allele number 23 and 57, respectively (Figure 3). Several virulence-related genes such as adhesins and flagellins were detected in these stains. All strains showed motility and lethality in hamsters similar to those seen with the RT027. RT883 showed spore production as higher as RT027, but the level of toxin production of RT027 were higher than all tested Brazilian strains.

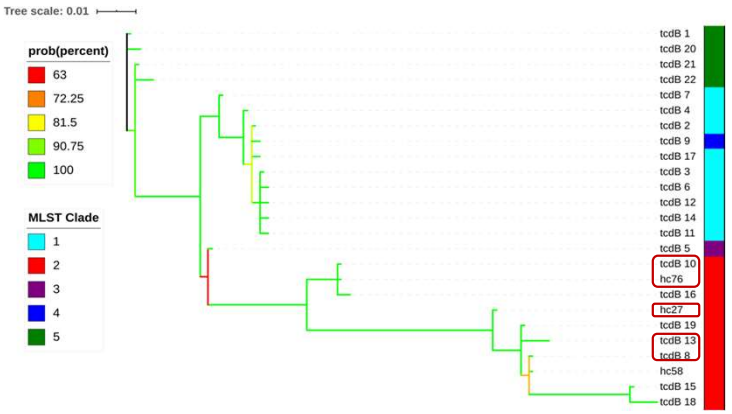

Figure 3: Bayesian phylogeny of *tcdB* genes, comparing with alleles in the literature using Mr.Bayes. In red is highlighted the alleles from strains RT883 (HC27), RT884 (HC58) and RT 885 (HC76)

All strains showed motility and lethality similar to those seen with the RT027 (Figure 4). RT883 showed spore production as higher as RT027, but the level of toxin production of RT027 were higher than all tested Brazilian strains.

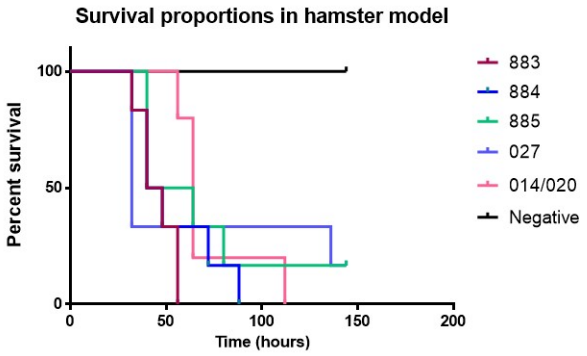

Figure 4. Kaplan-Meyer diagram showing the survival of hamsters (*Mesocricetus auratus*) of experimental groups over 8 days of observation.

CONCLUSIONS

In conclusion, the present study shows that the novel clade 2 strains circulating in Brazil have some phenotypic and genotypic hypervirulent characteristics and highlight the importance of surveillance studies to better known the epidemiology of *C. difficile* in Brazil.

REFERENCES

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