TITLE: ISOLATION OF *CLOSTRIDIOIDES DIFFICILE* STRAINS IN STOOLS OF DOMESTIC DOGS IN RIO DE JANEIRO, BRAZIL.

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ABSTRACT:

Clostridioides difficile is the major etiologic agent of nosocomial bacterial diarrhea associated with the use of antibiotics, and the production of cytotoxins (TcdA, TcdB and CDT) released by pathogenic strains is the main cause of the infections, commonly called CDI (*Clostridioides difficile* Infection). Since *C. difficile* has been isolated in animals, it has been suggested a potential zoonotic disease, and one of the routes of transmission that has been cogitated is in domestic animals, such as dogs and cats. Because gastrointestinal infections in dogs are still poorly understood, and the possibility of transmission to humans is suggested, this study aimed to isolate and characterize strains of *C. difficile* from dogs in Rio de Janeiro. Thus, samples of dogs stool (from 2 months to 18 years), with or without diarrhea, were randomly selected regardless gender or race distinction. Approximately, 0.5g of stool was inoculated in a differential medium (*Clostridioides difficile* Brucella agar - CDBA) and incubated under anaerobic condition at 37 ^{o}C. All colonies characteristic of *C. difficile* (resemble broken glass and Gram-positive rods) were confirmed by MALDI-TOF MS (BRUKER®) and also confirmed by polymerase chain reaction (PCR) using oligonucleotides for the species-specific gene (*tpi*- triose phosphate isomerase). A phenotypic characterization of the strains was performed through the observation of the susceptibility profile (Vancomycin, Metronidazole, Clindamycin) with tape E-test and assay for the biofilm production. Was also realized a genotypic characterization of the strains, trough a PCR to for the toxins genes (*tcd*A, *tcd*B and CDT), for the toxin-regulating gene - *tcd*C (partially sequenced for investigation of deletions), and for ribotype analysis. In total, 10% (5/50) of the strains were positive for *C. difficile*. Two of them were toxigenic (A^+B^+) and ribotyped as belonging to 106 ribotype. The other three strains were untypable. Considering the EUCAST breakpoints, all strains were sensitive to metronidazole and vancomycin. Those belonging to ribotype 106 were resistant to clindamycin, strong biofilm producers, cytotoxic and presented internal deletions in the *tcd*C gene sequence. None of the isolates were positive for the binary toxin genes. We believe that this study may contribute to the data referring to *C. difficile* strains and also contribute for treating enteric diarrhea in the veterinary medical clinic.

Keywords: *C. difficile,* zoonotic disease, dogs, genotypic characterization

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