Identification and characterization of *Clostridium difficile* strains isolated from dog feces in Rio de Janeiro

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Clostridium difficile is a gram- positive bacterium, in rod shape, and forming of spores. This pathogen is a major etiological agent of nosocomial bacterial diarrhea and the use of antibiotics predisposes susceptible patients to an imbalance of microbiota and colonization by *C. difficile*. The two primary cytotoxins released by pathogenic strains (TcdA and TcdB) are the cause of *Clostridium difficile* Infection (CDI) and, moreover, some strains are producing a binary toxin (CDT). By the fact of gastrointestinal infections in dogs are still poorly elucidated and the possibility of transmission to humans being suggested, the aim of this study was isolate *C. difficile* strains of dog feces, healthy or with diarrhea, in Rio de Janeiro. Thus, stool samples from 50 dogs, among these 10 diarrheal, were randomly selected without distinction of sex or race. All samples were kept frozen (-20°C) until they are needed. For the early identification, after an alcoholic shock, the samples were inoculated into BHI broth (0.1% sodium-taurocholate- ST) and kept in an incubator (37°C) under anaerobic conditions for at least 72h. After this period, the inoculum was performed in a differential medium (BHI-agar 0.0128 mg/L D-cycloserine; 500 mg/L cefoxitin and 0.1% ST) and the plates were incubated anaerobically for 48h at 37°C. All colonies characterized as *C. difficile* (colonies that resemble broken glass, Gram-positive and rod shape) had their identification confirmed by biochemical tests, the kit C diff Quick (Alere) and Maldi-TOF (Bruker). A genotypic confirmation by polymerase chain reaction (PCR) using primers for the species-specific gene *tpi* (triose phosphate isomerase) and toxins (TcdA and TcdB) were also performed. Of the 50 samples, 8 (16%) were confirmed as *C. difficile*. The stools of the positive animal owners for *C. difficile* were also processed, but the pathogen was not found. Of 8 positive samples, only one is toxigenic (PCR positive and C diff Quick complete). Most isolates were from animals with diarrhea and age ranging from 2 months to 5 years. A characterization of susceptibility profile (Vancomycin, Metroinidazol, Clindamycin, Erythromycin and tigecycline) with E-test strips is being conducted in addition to the characterization of the strains on the pulse type (PFGE) and ribotyping. We believe that this study will contribute to the data for the strains of *C. difficile*, their zoonotic potential and aid in the treatment of enteric diarrhea in animals.

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