

Título: ISOLATION AND CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* STRAINS AMONG FECAL SAMPLES FROM IMMUNOCOMPROMISED PATIENTS

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Resumo:

Clostridium difficile is a Gram-positive spore forming anaerobic bacterium, often associated with nosocomial diarrhea. The acquisition of this organism occurs primarily in hospitals, in its spore form, and its establishment and proliferation in the colon results from the removal of members of the normal intestinal flora during or after antibiotic therapy or even by the use of chemotherapeutic and immunosuppressive agents. The main virulence factors associated with pathogenic strains are toxins A and B and some strains produce binary toxin. This study aimed to isolate and characterize strains of *C. difficile* from stool samples of patients admitted to the Hematology, Oncology and Transplant wards at Hospital Universitário Clementino Fraga Filho (HUCCF / UFRJ). Between August 2013 and July 2014, 23 stool samples from 12 patients were collected. For immunological detection of toxins A and B from *C. difficile*, a ELISA kit (RIDASCREEN® *Clostridium difficile* Toxin A / B "(r-biopharm)) was used. Meanwhile, all fecal samples were subjected to alcohol shock and plated on CCFA medium for isolation of *C. difficile*. Three samples, two from the same patient, were positive for the presence of toxins A / B by ELISA. From these samples, it was possible to isolate three strains of *C. difficile*. Negative samples at ELISA test have not yielded *C. difficile* isolates. Identification was confirmed by PCR for the specie-specific gene, *tpi*. PCR was also used to test for toxins A, B and binary toxin genes. The strains isolated from the same patient were positive for toxins A and B genes. Both strains belong to ribotype 014/020. The remaining strain was shown to carry toxins A and B and the binary toxin gene. Sequencing showed a 36 bp deletion at the negative regulatory gene *tcdC*. This isolate belongs to a new ribotype not labeled yet. The antibiotic susceptibility was determined using E-test® strips. The strains were sensitive to metronidazole, vancomycin, teicoplanin, clindamycin and moxifloxacin and resistant to levofloxacin. Despite of the small sample size, we were able to identify a *C. difficile* strain belonging to a new ribotype. Most importantly, this is the first time that a binary toxin producer strain with *tcdC* deletion is detected in Brazil. Given the importance of this pathogen, the continuous monitoring of *C. difficile* and genetic/phenotypic characterization as well as the adoption of diagnostic routines are aspects that deserve attention in clinical laboratories.

Palavras-chave: *Clostridium difficile*, nosocomial infection, binary toxin

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