Short Report: *Clostridium difficile* in Adult Patients with Nosocomial Diarrhea in a Costa Rican Hospital

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Abstract. Stool samples from 104 adult patients with nosocomial antibiotic-associated diarrhea were analyzed for *Clostridium difficile* by cultivation, toxin A immunoenzymatic detection, and toxin B cytotoxic detection. The isolates were additionally screened for the toxin genes by polymerase chain reaction. *C. difficile* was isolated from 26 samples, and the toxins were directly detected in another 5 samples. In the toxigenic bacterial isolates, the detection rate of 30% indicates that *C. difficile* is a major etiologic agent of nosocomial diarrhea in Costa Rica.

The frequency of nosocomial diarrhea in patients receiving antibiotics ranges from 15% to 25%; this illness increases the morbidity and the mortality in hospitalized patients and elevates the costs for health systems. *Clostridium difficile* is the main etiologic agent due to the production of toxin A (TcdA), an enterotoxin, and toxin B (TcdB), a cytotoxin.

The diagnosis of *C. difficile*-associated diarrhea (CDAD) is based on a history of diarrhea associated with recent antibiotic use and tests to detect its toxins. Tissue culture assay is the “golden standard” but is technically challenging; therefore, immunoassays for toxin detection have been developed. Isolation is not sufficient to confirm CDAD and is less frequently used because of difficulties such as sensitivity to oxygen and nutritional requirements.

The incidence of CDAD has steadily increased in high-income countries, in part because of the awareness of its relevance. In low-income countries, however, there is scarce information concerning this etiologic agent. In Costa Rica, laboratory diagnosis for CDAD is not usually made; also, it is not mandatory to report its occurrence. This study was undertaken to assess its relevance in a major adult hospital in San José, Costa Rica.

The samples were collected during a 13-month period; one diarrheic stool sample was collected from each of 104 adult patients hospitalized for > 3 days who presented three or more loose, liquid, or watery stools in 24 hours. All patients were receiving one or a combination of the following antimicrobials: gentamicin (35%), cefotaxime and ceftazidime (20%, each one), and clindamycin, cephalexin, rifampicin, ciproxin, and oxacillin (16%, each one). No prescription pattern could be deduced from analysis of the clinical files.

To diagnose CDAD, three different approaches were undertaken: 1) *C. difficile* isolation, 2) immunoenzymatic detection of TcdA, and 3) cytotoxicity-based detection of TcdB. *C. difficile* were isolated on cycloserine-cefoxitin-fructose agar plates (CCFA; Oxoid, Cambridge, UK) as previously described and identified by the rapid ID 32A system (BioMérieux, Marcy l’Etoile, France). Negative samples were selectively enriched in prerduced chopped meat medium supplemented with c-cycloserine (250 mg/mL) and cefoxitin (8 mg/mL), incubated at 37°C for 72 hours and inoculated onto CCFA as previously described. TcdA was detected by the *C. difficile* TOX A TEST kit (TECLAB, Blacksburg, VA), according to the manufacturer’s instructions. TcdB was detected by a cytotoxicity assay using HeLa cells. Genomic DNA from the isolates was extracted by a phenol-chloroform procedure from a BHI culture. Primers for tcdA detection were as follows: NK11 (5′-TGATGCTAATAAGAATCCTAAAATGGA-3′) and NK9 (5′-CCACCAGCTGCAAGCCATA-3′); for tcdB detection were: NK104 (sequence-5′-GTGACATGAAGTCCAGGTTTAA-3′) and NK105 (sequence-5′-CACTTATCTCCATGTTGCGTGC-3′). These primers amplify a fragment of 1,200 bp from tcdA and a 200 bp fragment from tcdB.

Twenty-six samples were positive for the three detection methods, whereas five were positive for the toxin detection tests only (Table 1). Thus, 31 of the 104 patients suffered CDAD (30%). Non-toxigenic *C. difficile* were recovered from nine TdcA-negative samples: three of those samples induced non-specific cytotoxicity (Table 1).

The 26 isolates from samples positive for TcdA immunodetection assay and cytotoxicity were processed for detection of tcdA and tcdB; they were all positive for both genes. The other nine isolates were negative by polymerase chain reaction (PCR; Table 1).

This study is the first report of *C. difficile* in Costa Rican adult patients; the detection rate (30%) is similar to other reports in Ireland (27%), Argentina (36.8%), and Sweden (20%). This suggests that the prevalence of CDAD is not determined by the socioeconomic factors that distinguish high-income and low-income countries.

A good correlation between the detection methods was observed because 65% of the samples were positive by the three tests used. Detection of TcdA and TcdB in five stool samples without bacteria isolation reflects the fastidious character of *C. difficile*; the nine non-toxigenic *C. difficile* isolates correspond probably to intestinal indigenous bacteria. Other bacterial toxins have been previously described as responsible for non-specific cytotoxicity and may explain the toxicity observed in three samples that yielded non-toxigenic *C. difficile*.

In this work, 70% of the samples were negative for *C. difficile* and probably other agents were involved; recent stud-
ies have shown the role of enterotoxigenic strains of *C. perfringens* and *Bacteroides fragilis* in nosocomial diarrhea.17–19

This study confirms the relevance of *C. difficile* as a main etiologic agent of CDAD in a hospital of Costa Rica, responsible for at least 30% of the cases.

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