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Isolation of a *Clostridium* Exotoxin Producer Other than *Clostridium difficile* from a Patient with Diarrhea

Clostridium difficile-associated diarrhea is a significant problem in many hospitals and chronic care facilities (2). When established in the colon, pathogenic strains of *C. difficile* produce toxins that cause diarrhea and colitis; strains that do not produce toxins are not pathogenic. Toxin A (molecular mass, 308 kDa) acts as an enterotoxin, while toxin B (molecular mass, 250 to 270 kDa) acts as a cytotoxin (3), and both toxins may act synergistically in vivo.

We report a case of isolation of a *Clostridium* sp. other than *C. difficile* that produced A and B toxins from an elderly patient belonging to a group of 17 subjects with diarrhea who were admitted to the same room of the orthopedics unit and all treated with ceftriaxone for 7 to 10 days of prophylaxis before operation. Diarrhea in this group of patients lasted for 20 days, but the patients were not simultaneously affected. Stool samples from all patients were collected in clear containers and screened for exotoxins A and B by a commercially available enzyme immunoassay (Ridascreen *C. difficile* toxin A, B; R-Biopharm, Darmstadt, Germany). Swabs of stool specimen from each patient were transported in an anaerobic system (Port-A-Cul; BBL) and inoculated onto *C. difficile* selective agar plates (Oxoid) prepared in-house. The plates were then incubated anaerobically for 48 to 72 h at 35°C. Colonies were identified as *Clostridium* by colonial morphology, Gram's stain, and biochemical profile (API 20 A anaerobe and Vitek ANI systems; bio-Merieux).

Out of samples from 17 patients, toxins A and B were detected at different times in the stools of only 8 patients, but no *C. difficile* was isolated. In our experience, this is very unusual, because we generally isolate *C. difficile* from stools with toxins A and B. For the last 3 of the 17 patients we therefore considered all the clostridia isolated, namely, *C. paraputrificum*, *C. baratii*, *C. tertium*, *C. bifermentans*, *C. innocuum*, *C. clostridiiforme*, and *C. sporogenes*. After identification the clostridium strains were cultured and stored, before being checked for the presence of toxigenic products, in Bactec plus anaerobic vials (Becton Dickinson) containing liquid medium and matrix with fluorescent material that reacts to CO_2 produced by bacteria. This reaction, as measured by the photodetectors of an instrument, is evidence of bacterial growth. We chose this method because the bacterial culture is performed outside an anaerobic chamber and we observed (unpublished observation) that the production of bacterial exotoxins may be predicted by the black coloring of matrix at the bottom of the vials. Indeed, the bacterial cultures of nontoxigenic strains produced no change in color and the matrix remained brown, while exotoxin-producing strains changed the matrix to black. Only in one of the last three patients considered did we identify a strain of *C. baratii* producing exotoxins A and B. Cultures of this microorganism were positive for A and B exotoxin production by enzyme immunoassay and for cytotoxin B by tissue culture assay using Vero cells and *C. difficile* antitoxin B (Biolife) as a control. After the addition of a 0.5-ml inoculum (0.5 McFarland) in 30 ml of liquid medium, *C. difficile* ATCC 9689 produced black coloring of the matrix in 3 days while *C. baratii* produced black coloring in 8 days. This *Clostridium* sp. was positive for *C. difficile* antigens by a latex agglutination test (Meridian Diagnostics Inc.), but its biochemical profile and gas chromatography results were different from those of *C. difficile* ATCC 9689 and similar to those of a *C. baratii* toxin nonproducer. For toxigenic *C. baratii* tested by an agar dilution method (4), the MICs of teicoplanin and metronidazole were 1 and 8 mg/liter, respectively. The patients with diarrhea were treated at first with metronidazole without success and later with teicoplanin. This experience suggests that *Clostridium* microorganisms other than *C. difficile* sometimes produce A and B toxins but also that *C. baratii*, which is reported as a botulinal toxin producer (1, 5), is capable of toxin A and B production.

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