Concomitance of Cytotoxigenic and Non-Cytotoxigenic Clostridium difficile in Stool Specimens

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Six patients with antibiotic-associated diarrhea and one patient with diarrhea unrelated to antibiotic use yielded both cytotoxigenic and non-cytotoxigenic isolates of Clostridium difficile from the same stool specimens. In addition, these isolates were shown to be pathogenic and nonpathogenic, respectively, in the hamster model of antibiotic-associated colitis. These data imply that more than one toxin type of C. difficile may be harbored simultaneously. If toxin testing is used to identify C. difficile, more than one colony must be tested.

Clostridium difficile has been shown to cause pseudomembranous colitis (2, 6, 9, 14) and diarrhea (6, 7). In the majority of cases, a cytotoxin that is specifically neutralized by the cross-reacting Clostridium sordellii antitoxin and cytotoxigenic strains of C. difficile can be demonstrated in the stool specimens. Over recent years, a number of workers have described the isolation of non-cytotoxigenic strains from infants (12, 15), adults (6, 7, 11), animals (5), and the environment (5).

In these cases, where analyzed, no cytotoxin could be shown in stool specimens from humans. However, in two cases of carriage in animals, cytotoxin was present in the stool (5). We have noted six instances in which non-cytotoxigenic strains of C. difficile were isolated from the stool specimens of patients with antibiotic-associated diarrhea that also contained cytotoxin. It was thought that, although the strains failed to produce cytotoxin in vitro, the conditions in vivo were conducive to cytotoxin production. However, by the use of an isolator method described elsewhere (13), we were able to demonstrate that these isolates also failed to produce cytotoxin in vivo when administered to hamsters pretreated with clindamycin and housed individually. This finding prompted a more extensive investigation of these stool samples. Stools were reanalyzed for the presence of C. difficile by use of both a cycloserine-cefoxitin selective medium and alcohol to select for clostridial spores (4). Four serial 10-fold dilutions of the stool specimen were made in brain heart infusion broth. Samples of 0.1 ml were seeded onto the selective agar and, after treatment of the fecal suspension with alcohol, onto blood agar plates (4). All plates were incubated for 3 days at 37°C under anaerobic conditions. After incubation, at least 10 colonies of C. difficile from both sets of plates at the highest dilution were subcultured into chopped meat carbohydrate broth (10) and tested for the production of cytotoxin (3) after anaerobic incubation at 37°C for 3 days. In all cases, two cell lines, MRCV and Vero, were used for the detection of cytotoxin. These presumptive C. difficile isolates were further identified by fermentation of fructose, glucose, lactose, maltose, mannitol, mannose, melibiose, ribose, starch, and sucrose, by hydrolysis of esculin and starch (10), and by the detection of volatile fatty acid metabolic end products by gas-liquid chromatography after 3 days of growth at 37°C in chopped meat carbohydrate broth (10). In addition, growth of the isolates was inhibited by a strain of Clostridium beijerinckii, which is characteristic of C. difficile (1). In parallel with the testing of individual isolates, a large number of the C. difficile present were obtained by taking a sweep of the colonies at a low dilution on an agar plate by use of a wire loop and inoculating the bacteria into chopped meat carbohydrate broth, and also by the addition of stool directly to a selective broth as described by Chang and Gorbach (8).

For all seven cases, the stool specimens yielded isolates of C. difficile that differed in their ability to produce cytotoxin in vitro as detected by tissue culture and in vivo as determined by their inability to cause disease in the hamster model and by the absence of detectable levels of cytotoxin in the pellets of these colonized animals. For subject 6, cytotoxigenic isolates were only found after screening colonies from the lowest dilution plate. In all cases, both tissue culture cell lines used for the detection of cytotoxin gave identical results. Mixtures of toxin types were isolated by both isolation methods employed in all cases except for subject 6, for whom non-cytotoxigenic isolates were only ob-
tained by the alcohol spore selection technique. In all, four of the six samples on which a sweep technique was used were positive for cytotoxic production. All five of the samples tested by the method of Chang and Gorbach (8) were positive (Table 1), and this included one of the samples that was negative by the sweep technique. The failure to detect toxin in all cases by the sweep technique was probably due to growth inhibition of the cytotoxicogenic C. difficile by other bacteria that survived on the selective agar and subsequently multiplied when transferred to a nonselective broth.

These findings represent the first report of the isolation of both cytotoxicogenic and non-cytotoxicogenic isolates of C. difficile from the same stool samples from adults with non-antibiotic-associated and antibiotic-associated diarrhea (Table 1). In one case (subject 6), pseudomembranes were also present. The study also demonstrates that if toxin testing is used as a means of identifying C. difficile, it may be necessary to test more than one isolate if negative results are obtained.

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LITERATURE CITED