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Isolation of *Clostridium difficile* from Hospitalized Patients Without Antibiotic-Associated Diarrhea or Colitis

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Stool samples from 100 hospitalized patients and 21 healthy adults, obtained between March and June 1980, were cultured on a special selective medium containing cefoxitin and cycloserine to detect *Clostridium difficile*. This organism was isolated from 13 of the hospitalized patients and from 1 healthy subject. None of the patients with positive cultures had received antimicrobial therapy in the 3 preceding months. The observed rate of *C. difficile* isolation from adults not suffering from antibiotic-associated diarrhea or colitis is higher than previously reported. *C. difficile* culture is not recommended as a substitute for toxin assay in the evaluation of patients with intestinal disorders after antimicrobial chemotherapy.

The syndrome of antibiotic-associated colitis, especially the pseudomembranous variety, has been shown by numerous studies to be associated etiologically with *Clostridium difficile* (1–3, 6, 8, 15). This organism has the ability to proliferate when the bacteriological milieu of the intestine is altered. Enterotoxin is produced in large quantities under these circumstances, leading to mucosal damage and disease.

In clinical practice it is often desirable to obtain bacteriological confirmation of suspected cases, and a bioassay for *C. difficile* toxin has been successfully used for this purpose (6). This toxin has been found in 97% of cases of antibiotic-associated pseudomembranous colitis, in 27% of cases without pseudomembranes, and in only 2.5% of diarrheal conditions not associated with antibiotic usage (3). The toxin has also been found in 4% of patients with antibiotic-associated postoperative diarrhea (13), but it is not found in the feces of healthy adults (3). Unfortunately, the toxin assay depends on tissue culture techniques which are not available in most hospital laboratories. A counterimmunoelectrophoresis method has also been used for the detection of *C. difficile* toxin (18). This latter method, however, has not been as extensively tested as the tissue culture procedure. A possible alternative test for the diagnosis of *C. difficile*-associated colitis is the cultivation of this organism from fecal specimens. Positive cultures for this organism have been obtained in over 96% of toxin-positive stools (19), and a selective medium for the isolation of *C. difficile* has been devised (9). Using this medium which contains cycloserine, cefoxitin, fructose, and egg yolk (CCFA), we undertook a study to establish the prevalence of *C. difficile* in stools of a random population of hospitalized patients, many of them with diarrhea, and a group of normal adults.

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MATERIALS AND METHODS

C. difficile medium (CCFA) was purchased from the Regional Medical Laboratories (Remel, Lenexa, Kans.). Stool cultures from 100 hospitalized patients were obtained between March and June 1980. These cultures were requested by the attending physicians in their clinical work-up. The majority of patients had diarrheal problems, but in many the diarrhea was only a minor part of their overall disease. There were 36 male patients and 64 female patients. Four patients were less than 2 years of age; Six were children between 2 and 18 years of age, and the remaining were adults. The stools of 21 healthy adults (over the age of 18 and on no medication) were also obtained as controls. A very small quantity of undiluted stool (less than 0.1 g) was picked up between sterile swabs and plated directly onto the CCFA medium. The plates were incubated in an anaerobic chamber (Coy Anaerobic Chamber with a forced air incubator; Coy Laboratory Products, Ann Arbor, Mich.) for 48 h at 35°C. Suspected positive cultures showed the following features: (i) yellow, circular colonies (2 to 4 mm), flat to low umbonate, with or without filamentous edges; (ii) a change in the color of the surrounding medium from orange to yellow indicating fructose fermentation; (iii) absence of changes indicating lipase or lecithinase activity; (iv) chartreuse (yellow-green) fluorescence under long-wave UV light; (v) long, gram-positive rods in stained smears.

The suspected colonies were transferred to palladium chloride-containing reduced blood agar plates and incubated in the anaerobic chamber for another 24 h. This promoted the development of spores and filamen-

tous edges in the colonies. All suspected colonies were subcultured aerobically to rule out possible contamination by facultative fecal flora. Colonies fulfilling the above criteria were subcultured onto peptone-yeast extract-glucose broth for gas-liquid chromatography and into a modified Lombard-Dowell broth for biochemical testing with Minitek (BBL Microbiology Systems, Cockeysville, Md.) discs and plates (17). Tests included fermentation of glucose, mannose, mannitol, lactose, and sucrose and hydrolysis of esculin. All specimens were also plated onto Hecktoen enteric agar (BBL) and xylose-lysine-desoxycholate agar (BBL) and processed for the detection of salmonella and shigella. Eighteen of the stool specimens were inoculated onto Campy thio broth (Remel) and subcultured to Campy-agar (Remel) for the detection of *Campylobacter fetus* subsp. *jejuni* (4). The Campy-agar plates were incubated at 42°C in a candle jar containing a culture plate with *Providencia stuartii*, to create suitable O_2 and CO_2 concentrations by a modification of the Fortner principle (12). All stool specimens were also examined for ova and parasites which included concentration methods.

RESULTS

Of the 100 stools from hospitalized patients cultured, 13 yielded *C. difficile*. The organism also was isolated from one of the 21 normal subjects. Ten of ten organisms tested biochemically hydrolyzed esculin and fermented glucose and mannitol. None fermented lactose. Seven of ten also fermented mannose and sucrose. *C. difficile* is often reported as being incapable of fermenting sucrose. However, in one previous study (10), as in our series, a high rate of sucrose fermentation was found. Differences in methodology probably account for these variations. All 10 organisms tested produced major acetic and butyric acid peaks and minor isobutyric, isocaproic, and isovaleric acid peaks; 6 of 10 also produced minor valeric peaks, and two of ten produced a minor propionic peak. Biochemical testing and gas-liquid chromatography could not be done with four isolates that were lost in the process of subculturing. These four isolates were presumptively identified as *C. difficile* on the basis of their growth characteristics on CCFA and reduced blood agar medium, their chartreuse fluorescence, their ability to ferment fructose, and their microscopic morphology (subterminal spores). The *C. difficile* strains isolated in this study were not tested for toxin production.

Of 14 isolates identified as *C. difficile*, one was from a normal adult stool, three were from the stools of pediatric patients with diarrhea, two were from stools of patients with a history of inflammatory bowel disease, two were from stools of adult patients without diarrhea, and six were from stools of patients with diarrhea not associated with antibiotic therapy (Table 1). No other pathogens were isolated from the patients with positive *C. difficile* cultures. Other bacteria that grew on CCFA medium were studied morphologically only. They included budding yeasts, facultative long, thin curving gram-positive rods which produced colonies without fluorescence or filamentous edges, facultative gram-positive cocci in clusters, and facultative gram-positive rods.

TABLE 1. Intestinal pathogens isolated from 100 stool cultures in hospitalized patients and 21 healthy adults

Group	No. of patients	No. of patients from whom pathogens were isolated				
		C. difficile	*Salmonella* spp.	*Campylobacter* spp.[a]	*Giardia* spp.	Helminth ova
Adults with history of prior antibiotic treatment; does not include cases of inflammatory bowel disease	8	0	0	0	0	0
Adults without history of inflammatory bowel disease or antibiotic therapy	72	8	2	2	2	1[b]
Patients with inflammatory bowel disease (most of these patients received sulfasalazine)[c]	10	2	0	0	0	0
Infants and children with diarrhea	10	3	0	0	0	0
Healthy adults	21	1	0	0	0	0

[a] Only the last 18 samples were tested for *Campylobacter*.
[b] Four types of ova were isolated.
[c] Sulfasalazine (Azulfidine; Pharmacia) was given periodically to these patients. Duration of therapy ranged between 3 days and 5 years.

Other intestinal pathogens identified in stools that did not contain *C. difficile* included: *Campylobacter fetus* subsp. *jejuni*, 2 patients; *Salmonella* spp., 2 patients; *Giardia lamblia*, 2 patients; helminth eggs, 1 patient (Table 1). Only 18 stool specimens were checked for campylobacter since the procedure to isolate these organisms was instituted in the later part of the study.

DISCUSSION

Fecal cultures positive for *C. difficile* have been reported in a high proportion of patients with antibiotic-associated colitis, including some patients with negative toxin assays (2). On the other hand, this organism has also been found in fecal specimens in a variety of other circumstances. These include patients with idiopathic inflammatory bowel disease (5, 14), healthy infants (11, 16), and nearly 3% of healthy adults (8). In the present study, *C. difficile* was recovered from stool from 2 of 10 patients with inflammatory bowel disease (20%). The organism was also found in 3 of 10 pediatric patients with diarrhea (30%), in 8 of 72 hospitalized adults without a history of antibiotic usage within the preceding 3 months or of inflammatory bowel disease (11.1%). It was also found in 1 of

TABLE 2. *C. difficile* clinical correlation of 14 positive stool cultures

Case no.	Age	Sex	History of recent (3 mo) antimicrobial therapy	Clinical history	Proctoscopy
1	46 yr	F	None	Chronic diarrhea for 10 years (10 to 15 per day), spastic colon	Normal
2	43 yr	F	None	Spastic colon, frequent loose stools for 6 years	Normal
3	51 yr	F	None	Chronic diarrhea, post vagotomy syndrome	Not done
4	63 yr	F	None	Chronic psychophysiological diarrhea	Normal
5	78 yr	M	None	Mild diarrhea for 3 days, acute and chronic cholecystitis and cholelithiasis	Not done
6	95 yr	F	None	Gastrointestinal bleeding, colonic polyp, no diarrhea	No colitis
7	74 yr	F	None	Gastrointestinal bleeding, diverticulosis, hospitalized for mitral heart disease and atrial fibrillation, no diarrhea	Normal
8	24 yr	M	None	Acute abdominal pain, diarrhea, fever	Not done
9	1 mo	M	None	Diarrhea of short duration	Not done
10	18 mo	M	None	Diarrhea of short duration	Not done
11	4 yr	M	None	Diarrhea of short duration	Not done
12	56 yr	F	Sulfasalazine	Old history of idiopathic ulcerative colitis, recurrent episodes of diarrhea for 12 years	Normal
13	58 yr	F	Sulfasalazine	Crohn's disease for 6 years, diverticulosis, irritable bowel syndrome	Normal
14	25 yr	F	None	Healthy adult	Not done

21 healthy adults (4.8%). None of these patients had clinical evidence of pseudomembranous colitis.

Eight patients in this study received antimicrobial agents within 3 months before stool culture. The antimicrobial agents included ampicillin, clindamycin, tobramycin, vancomycin, erythromycin, cefamandole, cephalexin, penicillin, nystatin, and sulfonamide. *C. difficile* was not grown from the feces of any of these patients, although four of them had frequent loose stools. One of these patients, who received ampicillin for the treatment of bronchitis, developed an acute diarrheal episode and on proctoscopy was found to have mucosal erythema. This case may possibly represent antibiotic-associated diarrhea without pseudomembranes. Another patient was hospitalized with congestive heart failure and pneumonia, received clindamycin plus three other antimicrobials, and developed stool incontinence. A proctoscopic examination was not done on this patient, but clinically there was no suspicion of colitis. Diarrhea in a third patient was attributed to irritable bowel syndrome, whereas the fourth patient had only 2 days of mild diarrhea after treatment with ampicillin and tobramycin. Except for the first patient in this group, none of the patients had the clinical presentation of antibiotic-associated diarrhea or colitis. The clinical histories of patients who grew *C. difficile* in their feces were also carefully evaluated (Table 2). It is apparent from this that in most patients *C. difficile* did not play a pathogenic role and that the bacteriological findings were indicative of a rate of colonization higher than had been previously reported. We believe that the finding of this apparently increased rate of colonization is related to the high degree of sensitivity of the bacteriological techniques used. Falsen et al. (7), using similar techniques, have also demonstrated *C. difficile* in diarrheal patients without the typical antibiotic-associated colitis syndrome.

On the basis of our findings, we were convinced that culture is not a useful test for the bacteriological confirmation of *C. difficile*-induced antibiotic-associated colitis and suggest that toxin assay be used whenever such confirmation is clinically required.

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LITERATURE CITED

1. **Bartlett, J. G., T. W. Chang, M. Gurwith, S. L. Gorbach, and A. B. Onderdonk.** 1978. Antibiotic associated pseudomembranous colitis due to toxin producing clostridia. N. Engl. J. Med. **298**:531–534.
2. **Bartlett, J. G., N. Moon, T. W. Chang, N. Taylor, and A. B. Onderdonk.** 1978. Role of *Clostridium difficile* in antibiotic-associated pseudomembranous colitis. Gastroenterology **75**:778–782.
3. **Bartlett, J. G., N. S. Taylor, T. W. Chang, and J. A. Dzink.** 1980. Clinical and laboratory observations in *Clostridium difficile* colitis. Am. J. Clin. Nutr. **33**:2521–2526.
4. **Blaser, M. J., I. D. Berkowitz, M. LaForce, J. Cravens, L. B. Reller, and W. L. Wang.** 1979. Campylobacter enteritis: clinical and epidemiologic features. Ann. Int. Med. **91**:179–185.
5. **Bolton, R. P., R. J. Sherriff, and A. E. Read.** 1980. *Clostridium difficile* associated diarrhea: a role in inflammatory bowel disease. Lancet **i**:383–384.
6. **Chang, T. W., J. G. Bartlett, S. L. Gorbach, and A. B. Onderdonk.** 1978. Clindamycin-induced enterocolitis in hamsters as a model of pseudomembranous colitis in patients. Infect. Immun. **20**:526–529.
7. **Falsen, E., B. Kaijser, L. Nehls, B. Hygren, and A. Svedhem.** 1980. *Clostridium difficile* in relation to enteric bacterial pathogens. J. Clin. Microbiol. **12**:297–300.
8. **George, W. L., R. D. Rolfe, V. L. Sutter, and S. M. Finegold.** 1979. Diarrhea and colitis associated with antimicrobial therapy in man and animals. Am. J. Clin. Nutr. **32**:251–257.
9. **George, W. L., V. L. Sutter, D. Citron, and S. M. Finegold.** 1979. Selective and differential medium for isolation of *Clostridium difficile*. J. Clin. Microbiol. **9**:214–219.
10. **Hafiz, S., and C. L. Oakley.** 1976. *Clostridium difficile*: isolation and characteristics. J. Med. Microbiol. **9**:129–136.
11. **Hall, I. C., and E. O'Toole.** 1935. Intestinal flora in newborn infants with a description of a new pathogenic anaerobe *Bacillus difficilis*. Am. J. Dis. Child. **49**:390–402.
12. **Kaplan, R. L., and J. E. Barrett.** 1981. Monograph: campylobacter, p. 5. Marion Scientific, Kansas City.
13. **Keighley, M. R. B., J. Alexander-Williams, Y. Arabi, D. Youngs, D. W. Burdon, N. Shinagawa, H. Thompson, S. Bentley, and R. H. George.** 1978. Diarrhea and pseudomembranous colitis after gastrointestinal operations. Lancet **ii**:1165–1167.
14. **LaMont, J. T., and Y. M. Trnka.** 1980. Therapeutic implications of *Clostridium difficile* toxin during relapse of chronic inflammatory bowel disease. Lancet **i**:381–383.
15. **Mulligan, M. E., W. L. George, R. D. Rolfe, and S. M. Finegold.** 1980. Epidemiological aspects of *Clostridium difficile* induced diarrhea and colitis. Am. J. Clin. Nutr. **33**:2533–2538.
16. **Snyder, M. L.** 1940. The normal fecal flora of infants between two weeks and one year of age. J. Infect. Dis. **66**:1.
17. **Stargel, M. D., F. S. Thompson, S. E. Phillips, G. L. Lombard, and V. R. Dowell, Jr.** 1976. Modification of the Minitek miniaturized differentiated system for characterization of anaerobic bacteria. J. Clin. Microbiol. **3**:291–301.
18. **Welch, D. F., S. K. Menge, and J. M. Matsen.** 1980. Identification of toxigenic *Clostridium difficile* by counterimmunoelectrophoresis. J. Clin. Microbiol. **11**:470–473.
19. **Willey, S. H., and J. G. Bartlett.** 1979. Cultures for *Clostridium difficile* in stools containing a cytotoxin neutralized by *Clostridium sordellii* antitoxin. J. Clin. Microbiol. **10**:880–884.