

Clostridium difficile in Relation to Enteric Bacterial Pathogens

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All feces samples ($n = 2,390$) sent to the Bacteriological Laboratory, Göteborg, Sweden over 43 days were, in addition to the standard procedure, cultivated to detect *Clostridium difficile* by using a special selective medium. *C. difficile* was found in 81 of the 2,390 samples (3%). These 81 samples represented 56 patients. Fifty of the 56 patients had diarrhea. In 20 of the 56 patients (36%), *Salmonella*, *Campylobacter*, or *Yersinia* were also found. Of the 2,390 samples 252 (11%) from 132 patients revealed positive isolations of *Salmonella*, *Shigella*, *Campylobacter*, or *Yersinia* in comparison to 3% for *C. difficile* alone. This result suggests that *C. difficile* can easily be isolated with proper techniques. Concomitant isolations of more than one bacterial pathogen in cases of gastroenteritis were often found for *C. difficile*. The theory presented here is that any change of the normal bacterial fecal flora due to such causes as antimicrobial treatment or enteric infections like *Salmonella* increases the possibilities of isolating *C. difficile*. The causative significance of *C. difficile* might in most cases be doubtful. The majority of cases with diarrhea and *C. difficile* were self-healing and not severe. Only 2 cases of 56 had severe diarrhea with extended engagement of the colonic mucous membrane, but with no signs of pseudomembranes.

Diarrhea is commonly seen in patients treated with antimicrobial agents. This is often a reversible state and is caused by changes of the normal fecal flora.

A more serious form of diarrhea in connection with treatment by antimicrobial drugs is the so-called pseudomembranous enterocolitis (PMC), which has been described after treatment with tetracyclines, chloramphenicol, ampicillin, cephalosporins, and clindamycin (3, 10). Different bacteria, for example *Staphylococcus* spp., *Pseudomonas* spp., and *Proteus* spp., have been suggested to contribute to PMC (3). It has recently been suggested by Larson et al. (9) and Bartlett et al. (1) that, in many cases, toxin-producing clostridia can cause PMC. *Clostridium difficile* has especially been associated with PMC (2).

C. difficile is a gram-positive, obligate anaerobic rod, which has been considered somewhat difficult to isolate. It was described as early as 1935 by Hall and O'Toole (6) and was considered nonpathogenic for humans until recently (1, 9).

C. difficile toxin has been detected in feces from 98% of patients with PMC and in 15% of patients with antibiotic-induced diarrhea without signs of PMC (2). Epidemiological studies on healthy individuals indicate that *C. difficile* can be isolated from stools in 2% of adults (11).

The aim of the present investigation was to analyze all feces samples sent to the Bacteriological Laboratory, Göteborg, Sweden, in order

to study the frequency of *C. difficile* in Sweden in fecal samples and to correlate this with the occurrence of other intestinal pathogens and with clinical disease.

MATERIALS AND METHODS

Stool samples. All stool samples ($n = 2,390$) sent to the Bacteriological Laboratory, Göteborg, Sweden, over 43 days in October and November 1979 were included in the study and examined for *C. difficile*, *Salmonella* sp., *Shigella* sp., *Campylobacter fetus* subsp. *jejuni*, and *Yersinia enterocolitica*. The specimens were obtained with rectal swabs and sent to the laboratory in a transport medium (Stuart medium, slightly modified) especially well suited for anaerobic bacteria (12). Most specimens were plated within 8 h after collection.

Patients. Records from 42 of the 56 patients with isolation of *C. difficile* were analyzed retrospectively. Only scanty information was available for 14 of the patients.

Bacterial cultivation. The selective and differential CCFA medium was used for the primary isolation of *C. difficile* (5). Examination of the plates was performed after 48 h of incubation in anaerobic jars (6% CO₂, 94% H₂). Suspected colonies were subcultured on blood agar plates (5% horse blood). All isolates were submitted to Gram stain and gas-liquid chromatography analysis (7).

All isolates, as well as the reference strain ATCC 9689, are kept in the EF Culture Collection of this Institute.

Salmonella, *Shigella*, and *Yersinia* were cultivated

using standard techniques, and the selective medium of Skirrow was used for isolation of *C. fetus* subsp. *jejuni* (12).

RESULTS

C. difficile was isolated in 81 stool samples from 56 patients (22 females and 34 males). Of the 56 patients, 41 each had one stool sample positive for *C. difficile*, 8 had two samples positive, 6 had three samples positive, and 1 had six samples positive. The isolation frequency was 3% (81/2,390). The cases fell into three different age groups (Fig. 1). Children 4 years old or less were included in the first age group (43%, 24/56), and, within this group, 67% (16/24) were less than 1 year old. Of these, 31% (5/16) were children adopted from abroad.

The second age interval (39%, 22/56) consisted of young and middle-age adults between 15 and 44 years of age (Fig. 1). The third group (18%, 10/56) consisted of older people, 55 to 84 years of age.

A recent return from abroad was noted for 29%, 50%, and 10% of the individuals in the respective age groups (Table 1). Records were made available for 42 patients. In most cases diarrhea was the reason for having a stool culture performed. In at least 48% of the patients (20/42), treatment with antimicrobial agents was performed before enteritis developed. The distribution in the young, middle, and old age groups was 42, 18, and 60%, respectively (Table 1). Fourteen different antibiotics alone or in combination had been in use. Six patients received cotrimoxazole, five received phenoxymethylpenicillin, two received cephalothin, two received sulfonamide, and one each received flu-

cloxacillin, carbenicillin, ampicillin, cefoxitin, cefuroxime, neomycin, clindamycin, metronidazole, and pivmecillinam.

Diarrhea was seen in 50 of the 56 patients (89%). Only six individuals had stool cultivations performed as a routine health control procedure, without presenting symptoms of enterocolitis. Of these, four were adopted children. Two patients had a severe enterocolitic disease without pseudomembranes. One of these, who suffered from chronic lymphatic leukemia, was intensively treated with several antimicrobial agents for septicemia and died after 2 weeks. The other patient was given ampicillin treatment for pyelitis before his enterocolitis. The remainder of the patients had moderate diarrhea for a period of 1 to 2 weeks; only a few were proctoscoped, and none was colonoscoped.

Positive isolations were obtained in 168 patients. *Salmonella* and *C. difficile* were the two most common findings at 35 and 33%, respectively, followed by *Campylobacter*, *Shigella*, and *Yersinia* with 25, 15, and 8%, respectively. Concomitant isolations of other enteropathogens as distinct from *C. difficile*, *Salmonella*, *Campylobacter*, *Shigella*, and *Yersinia* are shown in Fig. 2. A selective isolation of only one intestinal pathogen was least common in the case of *C. difficile*, with 64%, followed by *Salmonella* and *Campylobacter* with 72 and 76%, respectively. The most frequent combination was *C. difficile* and *Salmonella*.

DISCUSSION

C. difficile has been considered difficult to isolate. However, the bacteria are easily recognized by a qualified microbiologist when selec-

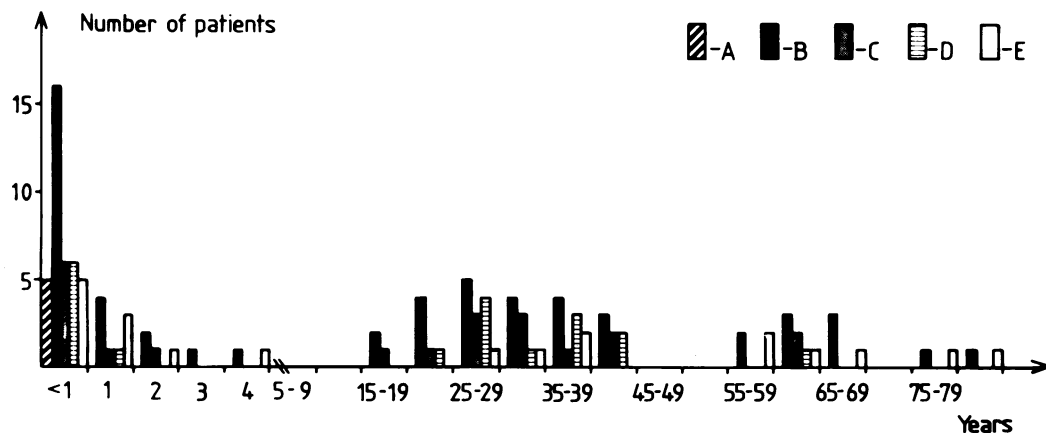


FIG. 1. Isolation of *C. difficile* from stools in the 56 patients. A, Children adopted from abroad; B, total number of patients; C, patients with concomitant isolation of gastrointestinal pathogens; D, patients recently returning from abroad; E, patients treated with antimicrobial agents before onset of enteritis.

tive medium is used. The characteristic odor of the bacterium is useful for the diagnosis. The bacteria were very vulnerable on the strongly selective medium, and all suspected colonies had to be subcultivated on nonselective media as soon as possible.

As judged from earlier reports, *C. difficile* can be isolated from stools from 2% of healthy individuals (11), which is approximately the isolation frequency in the present study. However, the majority of the patients from whom *C. difficile* was isolated did have diarrhea (50/56). Most patients had a moderate disease with mild diarrhea, and PMC was not identified in even the two patients with very severe diarrhea.

Antimicrobial treatment has been considered to induce PMC. In the present study, about 50% of the patients in whom *C. difficile* had been isolated had a recent period of antimicrobial treatment, but only 14% were treated with the antimicrobial agents reported to be especially correlated to PMC and the occurrence of *C. difficile*.

In the present study of patients with *C. difficile*, concomitant findings of intestinal bacterial pathogens were common. Our theory is, therefore, that PMC is a rare disease even in antimicrobial-treated patients developing diarrhea and in whom *C. difficile* is found. *C. difficile* might induce mild cases of diarrhea without pseudomembranes. Antimicrobial treatment or any gastrointestinal disorder where the normal bacterial flora of the intestines is changed seems to increase the possibilities for detection of *C. difficile*. In the present material, the patients in whom *C. difficile* was isolated were found in three age groups (Fig. 1). This finding is in agreement with the above-mentioned theory for the following reasons.

In small children and elderly patients, there was a high frequency of recent treatment with antimicrobial agents (42 and 60%, respectively), which influences the normal intestinal flora. In the age group from 15 to 44 years of age, antibiotic treatment was only administered in 18%

TABLE 1. Factors influencing the normal bacterial flora of the intestines^a

Influencing factor	Percent of age group (yrs):		
	0-4	15-44	55-84
Antimicrobial treatment prior to enteritis	42	18	60
Recent travel abroad	29	50	10
Concomitant isolation of bacterial pathogens	33	50	20

^a The figures are given in percent of each age group for 42 patients with isolation of *C. difficile*.

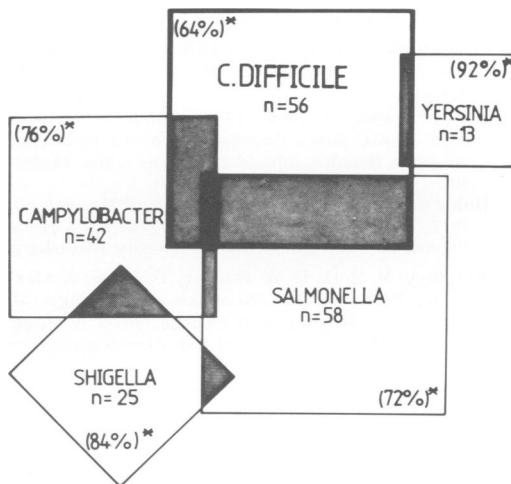


FIG. 2. Isolations of *C. difficile*, *Salmonella*, *Campylobacter*, *Shigella*, and *Yersinia* in 168 patients at the Bacteriological Laboratory, Göteborg, Sweden, over 43 days in October and November 1979. Parentheses indicate percentage of isolates of this species isolated where no other intestinal pathogens were found.

of the patients. In these individuals, however, concomitant findings of other intestinal bacterial pathogens were common: 50% as compared to 33 and 20%, respectively, of the individuals in the young and old age groups (Table 1). Furthermore, the middle age group had more often been recently abroad (50% as compared to 29 and 10%), which may cause a change in the normal intestinal flora (Table 1).

In conclusion, the mere finding of *C. difficile* in most cases of diarrhea without signs of PMC should not be an indication of treatment directed against *C. difficile*. In the rare cases of PMC, usually in compromised patients, where treatment is indicated, vancomycin seems at present to be the drug of choice (8).

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