

Selective and Differential Medium for Isolation of *Clostridium difficile*

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Clostridium difficile is a recognized cause of pseudomembranous (antimicrobial agent-associated) colitis and may be one of the causes of antimicrobial agent-induced diarrhea. A selective and differential agar medium that contains cycloserine, cefoxitin, fructose, and egg yolk (CCFA) was developed to facilitate the isolation of *C. difficile* from fecal specimens. Quantitative cultures of 16 stock strains of *C. difficile* on this medium (and on a medium containing cycloserine, fructose, and egg yolk) yielded counts equivalent to those obtained on blood agar; other media selective for clostridia, including Clostrisel agar, reinforced clostridial agar plus 0.2% *para*-cresol, and egg yolk-neomycin agar (the latter was inoculated with cultures subjected to prior heat shocking), were also tested and found to be inhibitory to the growth of *C. difficile*. Of 28 fecal or colostomy effluent specimens cultured on the above media, 14 yielded *C. difficile*. CCFA was found to be the most sensitive and selective of these media for the recovery of *C. difficile*. Colonies of *C. difficile* growing on CCFA had distinctive morphological and fluorescent properties which were sufficient for presumptive identification. CCFA should provide a rapid method for the screening of fecal specimens from patients with antimicrobial agent-associated diarrhea or colitis for *C. difficile*.

Toxicogenic isolates of *Clostridium difficile* have been demonstrated to be a major, if not the sole, cause of antimicrobial agent-induced ileocolitis of laboratory animals (2) and of pseudomembranous colitis of man (1, 4). *C. difficile* has also been shown to be the probable cause of diarrhea in one patient receiving ampicillin (1). These data have been summarized in a recent review (W. L. George, R. D. Rolfe, V. L. Sutter, and S. M. Finegold, *Am. J. Clin. Nutr.*, in press). Because the absolute count of *C. difficile* in feces may be an important determinant of whether an individual develops disease, of disease type (diarrhea versus pseudomembranous colitis) or of the severity of disease, we believe that a solid culture medium for the isolation of *C. difficile* from feces would be of value. It would presumably be useful in environmental studies also. To facilitate the diagnosis of pseudomembranous colitis and the study of its epidemiology, to assess the prevalence of *C. difficile* as part of the normal fecal flora, and to investigate the etiology of antimicrobial agent-associated diarrhea, we have developed selective and differential agar media for the isolation and presumptive identification of *C. difficile*. These media consist of an egg yolk-fructose base, to which either cycloser-

ine (medium designated CFA) or cycloserine and cefoxitin (medium designated CCFA) were added. Other media that have been reported to be selective for clostridia were included for comparison.

MATERIALS AND METHODS

The study was divided into two parts. In the first part, the inhibitory effect of several selective media upon stock strains of *C. difficile* was studied quantitatively. In the second part, fecal specimens from subjects who were thought likely to harbor *C. difficile* were quantitatively cultured.

Study of the inhibitory effect of selective media. (i) Microorganisms. A total of 16 isolates of *C. difficile*, identified by the methods outlined in the *Anaerobe Laboratory Manual* (8), were studied. Isolates of *C. difficile* numbered 1 to 12 were from the Wadsworth Anaerobic Laboratory collection (numbers 2112, 2994, 3065, 3490, 3657, 4139, 4265, 4266, 4268, 4269, 4277, and 4281). The sources of these isolates have been published previously (3). Isolates numbered 13 and 14 (WAL 4373 and 4374) had been recovered previously from cecal contents of mice that had received rosamicin. A. Onderdonk (Boston Veterans Administration Hospital) supplied isolate number 15 (BVA 17 HF 1-9) from a hamster with clindamycin-induced ileocolitis. Isolate number 16 was from the American Type Culture Collection (ATCC 9689).

(ii) **Media.** CFA and CCFA were prepared from an egg yolk-fructose agar base which consisted of 40 g of proteose peptone no. 2 (Difco Laboratories, Detroit, Mich.), 5 g of Na_2HPO_4 , 1 g of KH_2PO_4 , 2 g of NaCl , 0.1 g of anhydrous MgSO_4 , 6 g of fructose (ICN Pharmaceuticals, Inc., Cleveland, Ohio), 20 g of agar (Baltimore Biological Laboratory, Cockeysville, Md. [BBL]), and 3 ml of a 1% solution of neutral red in ethanol in 1,000 ml of distilled water. The base was dispensed in 100-ml portions, sterilized at 121°C and 15 lb/in² for 15 min, and stored aerobically at 4°C . Plates were prepared in the following manner. The above basal medium was melted and then cooled to 50°C , cycloserine base (500 $\mu\text{g}/\text{ml}$, final concentration; Eli Lilly & Co., Indianapolis, Ind.) and cofoxitin base (16 $\mu\text{g}/\text{ml}$, final concentration; Merck Sharp & Dohme Research Laboratory, West Point, Pa.) or cycloserine alone (500 $\mu\text{g}/\text{ml}$, final concentration) was added, and 5 ml of a 50% egg yolk suspension in saline (prepared in the laboratory) was added to yield CCFA and CFA, respectively. The container was swirled vigorously to ensure mixing, and portions of approximately 17 to 20 ml were poured into plastic petri dishes (15 by 100 mm).

Clostrisel agar (BBL) was prepared according to the directions of the manufacturer (without the addition of supplements).

Egg yolk-neomycin agar was prepared by adding neomycin base (100 $\mu\text{g}/\text{ml}$, final concentration) to egg yolk agar before autoclaving (11).

Reinforced clostridial agar with cresol was prepared by adding *p*-cresol (Baker grade; J. T. Baker Chemical Co., Phillipsburg, N.J.) at a final concentration of 0.2% (vol/vol) to melted reinforced clostridial agar (BBL) just before the plates were poured.

Brucella agar supplemented with hemin, vitamin K₁, and 5% sheep blood (11) was included for comparison with the selective media. All selective media, blood agar plates, and diluent were stored overnight in an anaerobic chamber before use.

(iii) **Inoculation.** An overnight growth of *C. difficile* in 10 ml of thioglycolate 135C broth (BBL), supplemented with 0.5 ml of peptic digest of blood (BBL), vitamin K₁, and hemin, was passed into the anaerobic chamber, blended in a Vortex mixer, and then diluted in a 10-fold series (10^1 to 10^7) in 0.05% yeast extract solution. CFA, CCFA, Clostrisel agar, reinforced clostridial agar with *p*-cresol, and blood agar plates were inoculated with 0.1 ml of each of the dilutions from 10^3 to 10^7 by a rotator-pipette method (H. R. Attebery and W. T. Carter, Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, E40, p. 11). The plates were placed in GasPak jars inside the anaerobic chamber, and the jars were sealed and then removed from the chamber for incubation. The 10^1 , 10^3 , 10^5 , 10^6 , and 10^7 dilutions were then placed in a water bath at 80°C for 10 min; egg yolk-neomycin agar plates were inoculated as described above, but under aerobic conditions, with 0.1 ml of the heated dilutions and placed in GasPak jars charged with GasPak generators. All plates were incubated at 37°C for 48 h.

(iv) **Colony counts.** Counts were performed on all plates with discrete colonies; the total count for each medium was expressed as log₁₀. Characteristics of *C. difficile* on the various media (colony size, morphol-

ogy, fluorescence) were noted.

Study of fecal specimens. A total of 26 fecal specimens from 25 subjects and 2 specimens of colostomy effluent were cultured for *C. difficile*. Pertinent clinical information, mode of transport of specimens, and interval from collection to processing of the specimen are given in Table 1.

Liquid specimens, which were all of small volume, were blended in a Vortex mixer with glass beads in a large glass screw-cap tube in the anaerobic chamber until visible homogeneity was achieved. Solid and semisolid specimens were mixed in a high-speed rotary blender for 3 to 5 min in the anaerobic chamber. After the specimen had been thoroughly mixed, a sample was removed, and serial 10-fold dilutions (10^1 to 10^9) were made in tubes containing 0.05% yeast extract and glass beads (to facilitate mixing).

Inoculation and incubation were performed by using the media and methods outlined above for the study of selective media. Inoculation of anaerobically stored egg yolk-neomycin agar plates with the 10^1 to 10^5 dilutions was done under aerobic conditions after heating at 80°C for 10 min in a water bath.

After 48 h of incubation, all plates were counted for total bacteria and for colonies which might be *C. difficile*. The small volume of most specimens prevented determination of dry weight. Counts of bacteria were therefore expressed as log₁₀ number of organisms per gram (wet weight) of stool.

Colonies of *C. difficile* growing on CFA, CCFA, and blood agar were examined under long-wavelength ultraviolet light (Mineralite UVSL-25; Ultraviolet Products, Inc., San Gabriel, Calif.) for fluorescence.

Whenever fluorescence, colonial morphology, or gram stain morphology resembled that of *C. difficile*, the isolate was identified by the criteria outlined in the *Anaerobe Laboratory Manual* (8). In addition, all other isolates that grew on CCFA were identified.

RESULTS

Study of the inhibitory effect of selective media. The results of the effects of various selective media upon growth of *C. difficile* in pure culture are presented in Table 2.

Counts of *C. difficile* on CFA and CCFA were not appreciably different from those on blood agar. Heating before inoculation of egg yolk-neomycin agar resulted in a 4 to 5 log₁₀ or greater decrease in counts. None of the isolates of *C. difficile* grew on the reinforced clostridial agar with *p*-cresol. Ten isolates failed to grow on Clostrisel agar; the remaining six isolates produced either a haze or pinpoint colonies on Clostrisel agar at lower dilutions only, making quantitative counts unfeasible.

Colony size was measured only on plates with fewer than 10 colonies to avoid the effects of crowding. Colony diameter of *C. difficile* averaged approximately 4.5 mm on blood agar and approximately 7.5 mm on CCFA after 48 h of incubation. *C. difficile* was found to have golden yellow fluorescence on CFA and CCFA and yel-

TABLE 1. Clinical information and specimen transport data

| Specimen no. | Patient data | | Conditions of storage and/or transport of specimens | |
|--------------|---------------------------------------|---|---|-------------------------------------|
| | Gastrointestinal disease | Antimicrobial therapy | Atmosphere | Interval to processing ^a |
| 1 | Diarrhea | Clindamycin, AG ^b | Anaerobic | ≤8 h |
| 2 | Diarrhea | Cephalothin | Anaerobic | ≤8 h |
| 3 | None ^c | None | Aerobic | ≤8 h |
| 4 | Loose stools | Penicillin V | Aerobic | ≤8 h |
| 5 | Pseudomembranous colitis | Penicillin G | Aerobic | 2 weeks |
| 6 | Pseudomembranous colitis ^d | Clindamycin, AG | Aerobic | 4 weeks |
| 7 | Neonatal necrotizing enterocolitis | Unknown | Aerobic | 6 months |
| 8 | Neonatal necrotizing enterocolitis | Unknown | Anaerobic | 2 days |
| 9 | Ulcerative colitis | Ticarcillin, AG | Aerobic | 5 months |
| 10 | Diarrhea ^d | Clindamycin, AG | Aerobic | 5 months |
| 11 | Diarrhea | Clindamycin, AG | Aerobic | 1 month |
| 12 | Pseudomembranous colitis | Clindamycin | Aerobic | 8 months |
| 13 | Pseudomembranous colitis | Clindamycin | Aerobic | 8 months |
| 14 | Diarrhea | Carbenicillin, cefazolin, AG | Aerobic | ≤8 h |
| 15 | Pseudomembranous colitis ^e | Cefazolin, cephalixin | Aerobic | 7 days |
| 16 | Pseudomembranous colitis ^e | Vancomycin | Aerobic | 3 days |
| 17 | Diarrhea | Clindamycin | Aerobic | 8 months |
| 18 | Pseudomembranous colitis | Tetracycline, erythromycin, chloramphenicol | Aerobic | 3 months |
| 19 | Pseudomembranous colitis | Unknown | Anaerobic | 9 days |
| 20 | Diarrhea | Ampicillin, clindamycin, AG | Aerobic | 2 days |
| 21 | Pseudomembranous colitis | Unknown | Aerobic | Unknown |
| 22 | Pseudomembranous colitis | Unknown | Aerobic | Unknown |
| 23 | Chronic diarrhea | None | Aerobic | 2 days |
| 24 | Diarrhea | Clindamycin, AG | Aerobic | ≤8 h |
| 25 | Diarrhea | Clindamycin, cephalirin | Anaerobic | ≤8 h |
| 26 | Diarrhea | Lincomycin | Aerobic | ≤8 h |
| 27 | None | Cefoxitin ^f | Anaerobic | ≤8 h |
| 28 | None | Cefoxitin ^f | Anaerobic | ≤8 h |

^a Indicates time interval between specimen collection and inoculation. Specimens which were not cultured within 8 h of collection were frozen at -70°C until processing.

^b AG, Aminoglycoside.

^c Specimen 3 was from a previously reported patient with clindamycin-associated pseudomembranous colitis (4) and was collected 4 months after complete recovery.

^d Specimen was colostomy effluent.

^e Specimens 15 and 16 were collected from the same subject before and during treatment with vancomycin.

^f Cefoxitin had been discontinued in both subjects approximately 1 week before specimen collection.

low-green or chartreuse fluorescence on blood agar after 48 h of incubation. Colonies of *C. difficile* growing on CFA and CCFA were yellow, of ground-glass appearance, circular with a slightly filamentous edge, flat to low umbonate in profile, and lipase and lecithinase negative. The initial orange color of the medium was often changed to yellow for 2 to 3 mm around the colony.

Study of fecal specimens. The numbers of *C. difficile* recovered on CFA and CCFA and the total counts of bacteria in the specimens are given in Table 3. *C. difficile* was not recovered from Clostrisels agar or reinforced clostridial agar with *p*-cresol; it was recovered from egg yolk-neomycin agar inoculated with heated dilutions

in one instance (specimen 28, count of 4.30 log₁₀ bacteria per g of feces) and from blood agar in two instances (specimens 15 and 16, counts of 3.90 and 3.00 log₁₀ bacteria per g of feces, respectively). The recovery of *C. difficile* from blood agar was made possible by demonstration of chartreuse fluorescence and subculture of the fluorescent area. Multiple subcultures were necessary, however, to isolate *C. difficile* in pure culture because growth of other organisms on the nonselective blood agar was either confluent or nearly confluent.

Microorganisms other than *C. difficile* frequently grew on CFA in large numbers, making isolation of *C. difficile* in pure culture difficult because of the necessity for repeated subculture.

TABLE 2. Growth of *C. difficile* on selective media

| Specimen no. | Growth on the following media: ^a | | | | | |
|--------------|---|------|------|-----------------|---|---|
| | Blood agar | CCFA | CFA | Clostrisel agar | Reinforced clostridial agar with cresol | Egg yolk-neomycin agar (heated dilutions) |
| 1 | 8.20 | 8.85 | 8.32 | NG ^b | NG | 3.18 |
| 2 | 7.95 | 8.04 | 8.00 | NG | NG | 2.85 |
| 3 | 8.08 | 8.48 | 8.30 | NG | NG | NG |
| 4 | 8.26 | 8.00 | 7.38 | NE ^c | NG | 4.74 |
| 5 | 8.28 | 8.60 | 8.30 | NG | NG | 4.48 |
| 6 | 8.30 | 8.48 | 8.11 | NE | NG | 4.32 |
| 7 | 8.32 | 8.23 | 8.70 | NG | NG | 4.08 |
| 8 | 8.32 | 8.00 | 8.20 | NE | NG | 4.00 |
| 9 | 8.38 | 8.08 | 8.26 | NG | NG | 4.00 |
| 10 | 8.40 | 8.20 | 8.26 | NG | NG | NT ^d |
| 11 | 8.51 | 8.70 | 8.70 | NE | NG | 3.56 |
| 12 | 8.30 | 8.30 | 8.08 | NG | NG | NG |
| 13 | 8.15 | 8.00 | 8.00 | NG | NG | NG |
| 14 | 8.04 | 8.00 | 8.00 | NG | NG | 3.67 |
| 15 | 8.08 | 7.80 | 7.84 | NE | NG | NG |
| 16 | 8.40 | 8.34 | 8.15 | ~7.48 | NG | NG |

^a Expressed as the highest absolute count (after correction for dilution) expressed as log₁₀ bacteria per ml.

^b NG, No growth.

^c NE indicates that counts could not be made because growth was either a haze or was >300 pinpoint colonies.

^d NT, Not tested.

C. difficile was the only organism that grew on CCFA from 8 of the 13 specimens that yielded *C. difficile* and were cultured on this medium. Organisms other than *C. difficile* that grew on CCFA were an unidentified anaerobic gram-negative bacillus (specimen 3), *Lactobacillus* sp. (specimens 5, 15, and 16), and an unidentified yeast (specimen 26). Colonies of these organisms were always small (pinpoint to 0.5 mm in diameter) and did not possess the characteristics of *C. difficile* (yellow fluorescence, low umbonate, filamentous edge, alteration of the color of the medium from orange to yellow).

Colonies of *C. difficile* from fecal cultures growing on CCFA were smaller (range, 1.5 to 9 mm; average, 4 mm) than those from pure cultures; the other gross morphological characteristics noted above for *C. difficile* in pure culture were retained, however, and *C. difficile* could readily be distinguished from the other organisms that occasionally grew on CCFA. In several instances, additional CCFA plates were inoculated and examined after 24 h of incubation. Colonies of *C. difficile* were easily detected after 24 h of incubation in counts similar to those observed on duplicate plates incubated for 48 h before examination.

Yellow fluorescence of colonies of *C. difficile* on CCFA could be detected after 24 h of incubation and persisted for 5 to 6 days. However, when these colonies were subcultured to supplemented brucella blood agar, the chartreuse fluorescence on blood agar was evanescent and

TABLE 3. Recovery of *C. difficile* from feces

| Specimen no. | Total bacterial count on blood agar | Count of <i>C. difficile</i> on CCFA | Count of <i>C. difficile</i> on CFA |
|--------------|-------------------------------------|--------------------------------------|-------------------------------------|
| 1 | 10.00 ^a | NT ^b | 7.00 |
| 3 | 10.78 | 3.30 | 0 ^c |
| 5 | 9.30 | 3.00 | 0 |
| 6 | 6.18 | 3.48 | 3.00 |
| 9 | 7.00 | 2.30 | 2.30 |
| 12 | 6.23 | 3.00 | 3.00 |
| 15 | 7.30 | 5.00 | 3.95 |
| 16 | 7.00 | 3.60 | 0 |
| 18 | 8.00 | 4.52 | NT |
| 21 | 8.23 | 7.00 | NT |
| 22 | 8.36 | 3.78 | NT |
| 26 | 9.00 | 4.38 | NT |
| 27 | 10.56 | 7.48 | NT |
| 28 | 9.04 | 6.15 | 0 |

^a All counts are expressed as log₁₀ number of organisms per gram (wet weight) of stool.

^b NT, Not tested.

^c A value of 0 indicates that *C. difficile* was not recovered on CFA, but CFA plates were overgrown with other bacteria.

could be consistently demonstrated only at 48 h of incubation.

Cells of *C. difficile* are gram-positive, usually 2 to 8 μm long by 0.5 μm wide, and possess subterminal to terminal spores (10). Cycloserine and cefoxitin were noted to alter the cellular morphology of *C. difficile*. When smears were made from colonies growing on CFA, elongation of the bacterial cells and a decrease in the num-

ber of spores were noted (compared with subsequent smears made from blood agar after subculture). Spores were never seen on Gram stains made from CCFA, and cellular elongation was even more marked than when the organisms were grown on CFA. The typical morphology of *C. difficile* was present after a single passage on blood agar, except for the isolates from specimens 27 and 28 (both subjects had recently received cefoxitin therapy); several passages on blood agar were required to demonstrate typical cellular morphology of the *C. difficile* isolated from these two specimens.

DISCUSSION

It is unclear why *C. difficile* failed to grow on the Clostrisel agar, reinforced clostridial agar with *p*-cresol, or egg yolk-neomycin agar. Bartlett et al. (2) and other investigators (9) have reported the use of Clostrisel agar to recover *C. difficile* from laboratory animals with clindamycin-induced ileocecolitis. However, the method of preparation of Clostrisel agar, the use of special additives (other than clindamycin), if any, and the length of incubation were not given. The use of reinforced clostridial medium (Oxoid Ltd., London, England) with 0.2% *p*-cresol as a selective broth medium to recover *C. difficile* from feces and the urogenital tract was reported by Hafiz et al. (5, 6). Larson (H. E. Larson, Clin. Res. 26:322A, 1978) reported that *C. difficile* isolated from cecal contents of hamsters with ileocecolitis would grow in 0.2% *p*-cresol broth. In a preliminary study, we found that the growth of stock strains of *C. difficile* was not inhibited by reinforced clostridial agar (L. George and D. Citron, unpublished data). When 0.2% *p*-cresol was added to this medium, the growth of all 16 stock strains of *C. difficile* was inhibited (Table 2). Thus, *p*-cresol at a concentration of 0.2% is too inhibitory to be used as a selective additive to a solid medium. When the concentration of *p*-cresol was reduced, the selectivity of the medium was lost.

The failure of *C. difficile* to grow from all but one specimen on egg yolk-neomycin agar inoculated with heated dilutions might have been due to several factors. Heat shocking of stock strains of *C. difficile* resulted in counts $4 \log_{10}$ or more lower than the counts when dilutions were not heated (Table 1). Only six of the fecal specimens studied had counts of *C. difficile* of $\geq 4 \log_{10}$; thus, *C. difficile* (initially present in counts of $4 \log_{10}$ or less) might not survive heat shocking. In addition, freezing of the fecal specimen might render *C. difficile* less viable.

Clindamycin-containing selective media have also been used to recover *C. difficile* from labo-

ratory animals with clindamycin-induced ileocecolitis (2) and from patients with diarrhea or pseudomembranous colitis associated with either ampicillin or clindamycin therapy (1). The use of a clindamycin agar to recover *C. difficile* from feces of patients with antimicrobial agent-induced diarrhea or colitis would be effective only when clindamycin-resistant *C. difficile* are responsible for the development of disease. We have reported previously on a patient who developed colitis 10 days after the cessation of therapy with clindamycin (4). The *C. difficile* recovered from his feces was susceptible to clindamycin (minimal inhibitory concentration, $<1 \mu\text{g/ml}$; minimal bactericidal concentration, $4 \mu\text{g/ml}$) and was not recovered on blood agar with clindamycin ($10 \mu\text{g/ml}$) added (3, 4). Moreover, 11 of 47 patients studied by Tedesco (12) developed symptoms of clindamycin colitis after cessation of clindamycin therapy. We have previously proposed that a sizeable proportion of cases of antimicrobial agent-associated colitis that develop after therapy might be due to antibiotic-susceptible (e.g., clindamycin-susceptible) *C. difficile* (George et al. Am. J. Clin. Nutr., in press). In such instances, the use of a selective medium that contains the offending antibiotic would likely be ineffective for the recovery of *C. difficile*.

The choice of cycloserine and cefoxitin as components of the medium was based on the level of resistance of 16 strains of *C. difficile* to cefoxitin (minimal inhibitory concentration, $\geq 32 \mu\text{g/ml}$) and to cycloserine (minimal inhibitory concentration, $\geq 1,024 \mu\text{g/ml}$) (3). The data from the study of selective media indicate that the growth of *C. difficile* (stock strains) was not inhibited by CCFA.

The low total counts of bacteria recovered from some of the specimens were probably due to several factors, including the dilutional effects of diarrhea and the reduction in the number of viable bacteria caused by freezing and/or prolonged storage at -70°C .

Specimens 13 and 19 (Table 3) were from patients with purported pseudomembranous colitis, yet *C. difficile* could not be recovered from either specimen, nor could toxin be demonstrated in tissue culture. Possibly these two cases of pseudomembranous colitis were not caused by *C. difficile*; however, loss of the organism and toxin as a result of improper specimen collection or freezing cannot be excluded.

High counts of *C. difficile* were recovered from the two subjects who had recently received cefoxitin (Table 3, specimens 27 and 28), yet neither of these patients had diarrhea or gastrointestinal complaints. Both isolates of *C. dif-*

difficile were toxigenic in tissue culture, yet toxin could not be detected in stool specimen 28 (George, Sutter, and Finegold, unpublished data). The significance of these data is unclear.

With CCFA, numbers of *C. difficile* as low as 2×10^3 in a total count of 6×10^{10} organisms could be detected. In most instances the only colonies growing on this medium were *C. difficile*. When other species of bacteria grew on CCFA, the distinction between them and *C. difficile* was easily made. Only *C. difficile* produced colonies which were greater than 0.5 mm in diameter, fluoresced golden yellow, and changed the color of CCFA from orange to yellow. Selectivity of CFA was less than that of CCFA; appreciable numbers of other bacteria grew on CFA, including some clostridia with yellow fluorescence (particularly *C. paraputrificum* and *C. perfringens*). The latter finding could be expected because cycloserine has been reported to be an effective selective agent for the isolation of these species of clostridia (7; D. J. Flora, C. R. Anselmo, V. L. Sutter, and S. M. Finegold, Abstr. Annu. Meet. Am. Soc. Microbiol. 1975, C40, p. 33). Optimal use of CCFA requires that colonies be picked after 24 to 48 h of incubation. When CCFA plates were reexamined after 3 to 6 days of incubation, significant numbers of colonies other than *C. difficile* had grown. The counts of *C. difficile* on CCFA were equal to or greater than the counts on CFA and blood agar in all instances. *C. difficile* could not be recovered on CFA from four of nine cultures so tested or on blood agar from 12 of 14 cultures. These data indicate that CCFA is an excellent selective and differential medium for the isolation of *C. difficile* from feces.

We have also found that in some instances *C. difficile* grows to a colony size of 2 to 4 mm after overnight incubation, thus enabling presumptive identification within 24 h after receipt of the specimen. CCFA should provide a relatively simple means for clinical and research laboratories to screen fecal specimens from patients with pseudomembranous colitis for *C. difficile*.

CCFA should also be useful for the investigation of ileocectitis of animals and for the survey

of environmental and human reservoirs for *C. difficile*. Because of its sensitivity, CCFA may also be of value in assessing the role of *C. difficile* in antibiotic-related diarrhea without pseudomembrane or plaque formation.

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