JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1985, p. 636–637
0095-1137/85/040636-02$02.00/0

Vol. 21, No. 4

Effect of Adding Sodium Taurocholate to Selective Media on the Recovery of *Clostridium difficile* from Environmental Surfaces

BRIAN P. BUGGY,*† CATHERINE C. HAWKINS,‡ AND ROBERT FEKETY

Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan 48109

Received 14 September 1984/Accepted 24 December 1984

The recovery of *Clostridium difficile* on a medium containing cefoxitin, cycloserine, fructose, and egg yolk was compared with that on media containing one of three preparations of sodium taurocholate. In aerobic environments contaminated with *C. difficile*, media containing either crude taurocholate from Mann Research Laboratories, New York, N.Y., or pure taurocholate from Sigma Chemical Co., St. Louis, Mo., recovered organisms significantly more often than did cefoxitin-cycloserine-fructose-egg yolk agar.

Cefoxitin-cycloserine-fructose-egg yolk agar (CCFA) is a selective medium used for the isolation of *Clostridium difficile* (3), the most important cause of antibiotic-associated colitis (1). This agar has been used for sampling the immediate environment of patients (6) and hamsters (7) with *C. difficile*-associated colitis. Substitution of 0.1% (wt/vol) sodium taurocholate for the egg yolk in CCFA (TCCFA) significantly increases the recovery of *C. difficile* spores from fecal specimens (9), whereas vegetative cells grow well on either medium (2, 9). Spores are the persistent form of this organism in an aerobic environment, as vegetative cells are viable only for minutes (2). In a previous study with an environmental surface model, Rodac plates (Becton Dickinson Labware, Oxnard, Calif.) containing crude sodium taurocholate (Mann Research Laboratories, New York, N.Y.) recovered 20 times as many *C. difficile* spores as did CCFA (2). The source and purity of sodium taurocholate affect the recovery of spores in vitro, with less pure taurocholate recovering fewer spores (8). These comparisons have all been made in laboratory settings. This study compared the use of these selective media for the recovery of *C. difficile* contaminating surfaces in aerobic environments.

Rodac plates were filled with ca. 17 ml of either CCFA or TCCFA containing one of three preparations of sodium taurocholate: a crude preparation from Mann Research Laboratories; a crude (40% pure) ox bile preparation from Sigma Chemical Co., St. Louis, Mo.; or a synthetic preparation (98% pure) from Sigma Chemical Co. Media were prepared as described previously (3, 9). Paired (side-by-side) impression cultures (one plate of the pair containing CCFA and the other plate containing one of the preparations of TCCFA) were done for samples collected from multiple sites during three experiments. (i) CCFA and crude Mann TCCFA were used to culture samples from surfaces in the hospital rooms of 10 patients with *C. difficile*-associated colitis in a manner previously described (6); (ii) CCFA and crude Sigma TCCFA were used to culture samples from the cage surfaces of hamsters with antibiotic-induced *C. difficile* colitis (4); and (iii) CCFA and pure Sigma TCCFA were used in a later part of this study to culture samples from hamster cages. Therefore, two cultures were done for each site (one with CCFA and the other with one of the taurocholate preparations). Each medium was assumed to have an equal chance to recover any *C. difficile* colonies present at the site. Isolates were identified as *C. difficile* by typical colony morphology and standard biochemical and gas-liquid chromatographic methods (5). Results were analyzed by chi-square analysis.

Table 1 shows that 177 of 567 (31%) sites were culture positive. When only one plate of the paired cultures recovered *C. difficile* from a given site (53% of all positive sites), significant differences were seen among the media. Both crude Mann ($P < 0.5$) and pure Sigma ($P < 0.01$) TCCFA recovered *C. difficile* more often than did CCFA. Only crude Sigma TCCFA yielded significantly fewer positive cultures than did CCFA ($P < 0.001$). These results are consistent with previous in vitro data concerning the ability of taurocholate to recover *C. difficile* spores (2, 8).

Both plates of the paired cultures were positive for 47% of the environmental sites. The mean number of colonies per Rodac plate varied in each experiment (from one to confluent growth). There was no significant difference in colony counts per plate between CCFA and taurocholate-containing media when both cultures were positive for a given site.

If media containing sodium taurocholate are used for the environmental detection of *C. difficile*, the source and purity of the taurocholate should be noted. Mann Research Laboratories no longer manufactures crude taurocholate. Other bile salt preparations have been evaluated for their ability to enhance the germination of *C. difficile* spores in vitro (8), but none have been tested during environmental sampling under aerobic conditions.

Although the difference between CCFA and TCCFA in recovering *C. difficile* was not as large as that seen in previous in vitro experiments (2, 8, 9), both crude Mann and pure Sigma TCCFA recovered *C. difficile* contaminating

TABLE 1. Environmental recovery of *C. difficile*

Study	Taurocholate prepn	No. of sites positive on: CCFA only	TCCFA only	Both agars	Total no. of positive cultures/total no. of sites cultured (%)
Hospital	Mann (crude)	12	22	10	44/117 (38)
Hamster	Sigma (crude)	33	8	25	66/264 (25)
Hamster	Sigma (pure)	3	15	49	67/186 (36)

* Corresponding author.
† Present address: Department of Medicine, Medical College of Wisconsin, and St. Luke's Hospital, Milwaukee, WI 53215.
‡ Present address: Division of Infectious Disease, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

aerobic environmental sites significantly more often than did CCFA.

This work was supported by grants from The Upjohn Co. and the Baker Infectious Diseases Research Fund of the University of Michigan and by Public Health Service grant AM-21076-03 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

We thank Kenneth Wilson for review of the manuscript, Susan Seo for technical assistance, and Marlys Boyer for secretarial support.

LITERATURE CITED

1. **Barlett, J. G.** 1979. Antibiotic-associated pseudomembranous colitis. Rev. Infect. Dis. **1**:530–539.
2. **Buggy, B. P., K. H. Wilson, and R. Fekety.** 1983. Comparison of methods for recovery of *Clostridium difficile* from an environmental surface. J. Clin. Microbiol. **18**:348–352.
3. **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.
4. **Hawkins, C. C., B. P. Buggy, R. Fekety, and D. R. Schaberg.** 1984. Epidemiology of colitis induced by *Clostridium difficile* in hamsters: application of a bacteriophage and bacteriocin typing system. J. Infect. Dis. **149**:775–780.
5. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
6. **Kim, K.-H., R. Fekety, D. H. Batts, D. Brown, M. Cudmore, J. Silva, Jr., and D. Waters.** 1981. Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. J. Infect. Dis. **143**:42–50.
7. **Toshniwal, R., J. Silva, Jr., R. Fekety, and K.-H. Kim.** 1981. Studies on the epidemiology of colitis due to *Clostridium difficile* in hamsters. J. Infect. Dis. **143**:51–54.
8. **Wilson, K. H.** 1983. Efficiency of various bile salt preparations for stimulation of *Clostridium difficile* spore germination. J. Clin. Microbiol. **18**:1017–1019.
9. **Wilson, K. H., M. J. Kennedy, and R. Fekety.** 1982. Use of sodium taurocholate to enhance spore recovery on a medium selective for *Clostridium difficile*. J. Clin. Microbiol. **15**:443–446.