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

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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

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<th>TABLE 1. Environmental recovery of C. difficile</th>
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<td>Study Taurocholate prep</td>
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</tr>
<tr>
<td>Hospital Mann (crude)</td>
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<tr>
<td>Hamster Sigma (crude)</td>
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<td>Hamster Sigma (pure)</td>
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aerobic environmental sites significantly more often than did CCFA.

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LITERATURE CITED