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Comparative Study of Selective Media for Isolation of *Legionella pneumophila* from Potable Water

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A total of 100 water samples, 95% of which were taken from hospital potable water fixtures, were cultured on three different media used for the isolation of *Legionella pneumophila*. The media used were buffered charcoal-yeast extract medium (BCYEα medium), BCYEα medium with antimicrobial agents (BMPAα medium), and BCYEα medium with antimicrobial agents, glycine, and differential dyes (MWY medium). An acid wash procedure was also used for specimens plated on BCYEα and BMPAα media. A total of 24 samples were culture positive for *L. pneumophila* by one or more techniques. MWY medium detected 92% of positive cultures, BCYEα medium with the acid wash detected 83% of positive cultures, BMPAα medium detected 79% of positive cultures, BCYEα medium detected 71% of positive cultures, and BMPAα medium with the acid wash detected 62% of positive cultures. MWY medium was the best medium for isolating *L. pneumophila* from potable water specimens and can probably be depended upon as the sole medium for this type of testing.

Detection of *Legionella pneumophila* in potable water specimens is needed in investigations of Legionnaires disease when a potable water source is epidemiologically implicated (7, 8). It has previously been determined in this laboratory that selective media for this purpose are more effective than guinea pig inoculation (5). The relative sensitivities of some types of selective media have also been studied in this laboratory; these studies led to the conclusion that a set of four plates, with two different media and an acid wash technique, was needed for optimal yield (3). Wadowsky and Yee have recently developed a new selective medium (WY medium) specifically designed for the isolation of *L. pneumophila* and *L. micdadei* from potable water sources (9). This study, in which a modification of WY medium (MWY medium) was used, examined prospectively the relative sensitivity of MWY medium versus that of the other media and techniques previously used in this laboratory.

MATERIALS AND METHODS

Media. Three different media were studied: buffered charcoal-yeast extract medium supplemented with 0.1% α-ketoglutarate (BCYEα medium); BCYEα medium supplemented with 4 μg of cefamandole, 80 U of polymyxin B, and 80 μg of anisomycin per ml (BMPAα medium); and BCYEα medium supplemented with 0.3% glycine, 50 U of polymyxin B, 1 μg of vancomycin, 80 μg of anisomycin, 10 μg of bromthymol blue, and 10 μg of bromcresol purple per ml (MWY medium) (3, 9). BCYEα and BMPAα media were made as previously described (3). MWY medium is based on the glycine-containing medium (WY medium) described by Wadowsky and Yee (9); it differs in that WY medium contains 5 μg of vancomycin and 100 U of polymyxin B per ml: WY medium does not contain anisomycin or α-ketoglutarate, which were added to inhibit yeasts and to improve the growth of *L. pneumophila*, respectively (3). In addition to the three media, an acid wash technique was used with BCYEα and BMPAα media (1, 3). The acid wash technique was not found to enhance the recovery of *L. pneumophila* on MWY medium in a preliminary study, so it was not used with this medium in the prospective study.

Specimen collection and processing. A total of 100 consecutively collected water samples from this hospital were tested prospectively. Of the 100, 95 were taken from potable water sources and fixtures; both chlorinated (free residual chlorine, 2 to 4 mg/liter) and nonchlorinated (free residual chlorine, <0.1 mg/liter) samples were studied. The other five samples were cooling tower water samples. All samples were collected in sterile containers containing sodium thiosulfate at a final concentration (after collection) of 200 μg/ml. Sample volumes ranged from 100 ml to 40 liters. Large-volume samples were often concentrated by continuous flow or conventional centrifugation before testing. Eighty percent of the samples were 100-ml samples, containing both water and pipe or fixture sediment, obtained by swabbing with moistened sterile cotton swabs (3, 5).

Plating of samples was done within 24 h of collection; specimens not plated immediately were refrigerated at 5°C until processed. Five portions of each sample were taken after thorough mixing. Three of these were plated directly on BCYEα, BMPAα, and MWY media; 0.1 ml was inoculated on each plate, which was then streaked for isolation. The same volume of a 1:10 dilution of the sample in HCl-KCl acid wash solution was also plated onto separate BCYEα and BMPAα plates in accordance with previously described techniques (1, 3). Plates were incubated for 14 days in a humidified air incubator at 35°C with 90% relative humidity.

Organism identification. Plates were inspected daily; only colonies resembling *Legionella* spp. were picked (6). This was facilitated by a dissecting microscope. Special attention was paid to colony color on MWY medium, as this is a differential characteristic of the medium (9). On day 14, all plates were examined with long-wave UV light (366 nm) to detect those *Legionella* spp. which fluoresce blue-white and which may have been missed earlier (6). *Legionella* spp. were identified on the basis of growth and biochemical and immunological characteristics (6).

Statistical analysis. The null hypothesis examined was that the yield of positive cultures was not significantly different with MWY medium than with the other four (standard) techniques; this was tested by a McNemar two-tailed nonparametric statistical test (2).

RESULTS

A total of 24 specimens were culture positive for *L. pneumophila*; serogroups 1 and 4 were detected. No other species of *Legionella* or serogroups of *L. pneumophila* grew. Of these 24 positive specimens, 22 (92%) were positive on MWY medium, 20 (83%) were positive on BCYEα medium plated with acid-washed samples, 19 (79%) were positive on BMPAα medium plated with non-acid-washed samples, 17 (71%) were positive on BCYEα medium plated with non-acid-washed samples, and 15 (62%) were positive on BMPAα medium plated with acid-washed samples. One positive sample grew only on MWY medium, and two positive samples failed to grow on MWY medium. Two of the discordant sample results were from specimens which yielded three or fewer colonies of *L. pneumophila* on the positive plate; a sampling error may account for the difference. The other discordant sample failed to grow on MWY medium because of overgrowth of a mold; *L. pneumophila* was recovered from this sample by plating on BMPAα medium with and without acid treatment and on BCYEα medium with acid treatment. No significant difference in serogroup yield was detected on the various media.

Statistical analysis of the data confirmed the null hypothesis, that is, that yield with MWY medium was not significantly different from yield with the standard techniques combined ($P > 0.5$).

DISCUSSION

This study indicates that MWY medium is a sensitive medium for detecting *L. pneumophila* in potable water samples. Since plating on MWY medium alone would have led to two false-negative specimens (one of which was probably caused by a sampling error), it is possible that some studies may require the use of other techniques. However, the use of standard techniques alone would have led to one false-negative specimen; as demonstrated in the statistical testing, this is not a significant difference. Therefore, use of MWY medium alone can justifiably be advocated, especially when multiple samples are tested. Plating in duplicate or increasing inoculum volume may increase yield further. As discussed in a previous paper, these results may hold only for potable water samples and only for the recovery of *L. pneumophila* (5). It is very likely that *L. micdadei* yield will be higher on MWY medium than on BMPAα medium, as BMPAα medium inhibits *L. micdadei* (unpublished data). I do not know if the results observed in this study would have been the same if unmodified WY medium had been used instead of MWY medium, but I suspect that the yield would be lower because of overgrowth by some yeasts and because of poorer growth of *L. pneumophila* without added α-ketoglutarate and in the presence of higher concentrations of vancomycin (3, 4).

I therefore suggest that MWY medium alone be used for the detection of *L. pneumophila* in potable water samples when such sources are incriminated by epidemiological investigations of outbreaks of Legionnaires disease.

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