APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1978, p. 771-773
0099-2240/78/0036-0771$02.00/0

Vol. 36, No. 5
Printed in U.S.A.

# NOTES

## Use of Cetylpyridinium Chloride in the Decontamination of Water for Culture of Mycobacteria

GARY C. DU MOULIN[1]* AND KURT D. STOTTMEIER[2]

*Department of Anaesthesia, Harvard Medical School at Beth Israel Hospital, Boston, Massachusetts 02215,[1] and Massachusetts Mycobacteria Reference Laboratory, Mattapan Hospital, Boston, Massachusetts 02126[2]*

Received for publication 31 July 1978

Selective decontamination of large-volume aqueous samples for mycobacterial culture can be achieved with overnight exposure to 0.04% cetylpyridinium chloride.

Water can be a source for potentially pathogenic mycobacteria (2). These organisms have been frequently isolated from environmental sources, including residential and hospital taps, springs, cisterns, and drinking fountains (2, 9). Recovery of mycobacteria from patient material with subsequent identification of identical organisms from the hospital environment suggests that drinking water is an important mode of transmission (1, 3). Suggestions for microbiological examination of water in recognized manuals do not contain a standardized protocol for the recovery of mycobacteria. Methods used for water are similar to those used for the processing of sputum and urine (7, 8). These procedures are not optimal for the culturing of water for acid-fast organisms for three reasons. First, the processing of clinical material is usually carried out on specimens containing relatively small numbers of non-acid-fast organisms. Depending upon the source, water specimens from the environment may contain abundant bacterial as well as fungal species. This leads to difficulty in attaining adequate growth suppression of these contaminating organisms. Second, adequate culturing of natural or finished waters necessarily requires the collection of large specimens in which concentration of the organisms is achieved by membrane filtration. Filters are generally composed of either mixed esters of cellulose or polycarbonate film which is sensitive to the alkaline pH used for decontamination of mycobacterial specimens. Third, alkali treatment of water-borne mycobacterial organisms may in itself suppress the growth of these organisms and decrease the chances of recovery.

The use of cetylpyridinium chloride was found to effectively replace the alkali decontamination procedures of specimens for mycobacterial analysis and seems especially suited for decontamination of large volumes of water.

Cetylpyridinium chloride has been recommended as a means of eliminating non-acid-fast organisms from sputum (6, 10, 11) and was recently applied to the decontamination of mouse footpads after experimental infection with *Mycobacterium ulcerans* (4). Cetylpyridinium chloride was also utilized to decontaminate gastrointestinal contents and fecal specimens after experimental alimentary tract infection of lizards with *M. ulcerans* (5).

Cetylpyridinium chloride (cetylpyridinium chloride monohydrate; Aldrich Chemical Co., Milwaukee, Wis.), a detergent, is added to the water specimen to a final concentration of 0.04%. The specimen is thoroughly mixed and allowed to stand at ambient temperatures for 24 h. The treated specimen is filtered through bacteriological membrane filters. We use a stirred model 402 ultrafiltration cell fitted with 76-mm, 0.45-$\mu$m microporous (cellulose acetate) filters (Amicon Corp., Lexington, Mass) as a filter support; however, other similar filtration devices can easily be employed. Other membrane filter compositions can be used as well, although polycarbonate filters (Nucleopore Corp., Pleasanton, Calif.) were difficult to handle aseptically, and the dark appearance of the filter on media tended to obscure early colonial development. After subsequent washing of membranes with 500 ml of sterile distilled water to remove residual detergent, the filter is aseptically transferred to appropriate media for the recovery of mycobacteria. Plates are protected from desiccation and incubated at 37°C under 5 to 10% $CO_2$ for 60 days to allow for growth.

Survival of mycobacteria and water-associated potential pathogens after contact with cetylpyridinium chloride was studied. Samples (1,000 ml) of sterile distilled water were seeded with dilutions of 5-day-old cultures of atypical mycobacteria grown in Middlebrook 7H9 broth. The organisms used were originally obtained from clinical isolates and represented the four groups of the Runyon classification system, specifically, *Mycobacterium kansasii, Mycobacterium gordonae, Mycobacterium intracellulare,* and *Mycobacterium fortuitum.* A strain of *Mycobacterium bovis* (BCG) was also tested for detergent susceptibility. Four strains of water-associated gram-negative bacilli were included: *Pseudomonas aeruginosa, Pseudomonas cepacia, Flavobacterium* sp., and *Acinetobacter calcoaceticus.* After detergent treatment and filtration, filters containing mycobacteria were transferred to Middlebrook-Cohn 7H10 agar plates (100 by 15 mm; Gibco Diagnostics, Madison, Wis.). Filters containing gram-negative bacilli were placed on plates (100 by 15 mm) containing Trypticase soy agar with 5% sheep blood agar (Scott Laboratories, Fiskeville, R.I.). All plates were individually covered with plastic and incubated for 60 days in candle jars at 37°C. Controls consisted of an identical series of specimens which underwent no detergent treatment. Results in the form of viable units per 1,000 ml are shown in Table 1.

Decontamination with cetylpyridinium chloride of environmental water specimens for mycobacterial culture was carried out in a similar manner. After filtration and washing of the membranes, filters were transferred to Middlebrook-Cohn 7H10 agar plates (100 by 15 mm; Gibco Diagnostics). The plates were sealed in individual plastic bags to retain moisture and incubated at 37°C without $CO_2$ for 60 days. Weekly inspection of plates was made until growth became evident. Ziehl-Neelson stains could then be carried out on isolates with subsequent standard biochemical identification (12).

Of 54 1-liter environmental water samples from hosptial water supplies (obtained from central pump room pumps, shower heads, bedside carafes, and nebulizer reservoirs) processed in this manner, 29 (52%) were positive for mycobacteria (25 positive for *M. chelonei* and 9 positive for *M. intracellulare*), 20 were negative for mycobacteria, and 5 became contaminated with molds. *M. chelonei* appeared within 7 days. *M. intracellulare* required approximately 3 weeks. All plates were incubated for 60 days; however, after initial appearance there was no increase in numbers of colonies, only colonial size.

TABLE 1. *Effect of cetylpyridinium chloride on water specimens seeded with acid-fast and non-acid-fast bacteria*

| Bacterial species | Untreated (viable units/1,000 ml) | Treated (viable units/1,000 ml) | % Survival |
|---|---|---|---|
| *P. aeruginosa* | $1.3 \times 10^3$ | No growth | 0 |
| *P. cepacia* | $4.1 \times 10^3$ | No growth | 0 |
| *Flavobacterium* sp. | $1.9 \times 10^3$ | No growth | 0 |
| *A. calcoaceticus* | $4.3 \times 10^3$ | No growth | 0 |
| *M. kansasii* | $4.4 \times 10^3$ | $8.1 \times 10^2$ | 18.4 |
| *M. gordonae* | $1.7 \times 10^4$ | $1.4 \times 10^3$ | 8.4 |
| *M. intracellulare* | $4.4 \times 10^2$ | $7.4 \times 10^3$ | 100.0 |
| *M. fortuitum* | $2.3 \times 10^3$ | $2.6 \times 10^1$ | 1.1 |
| *M. bovis* (BCG) | $5.5 \times 10^3$ | $2.2 \times 10^3$ | 39.9 |

Under the treatment criteria described above, non-acid-fast bacteria were effectively destroyed. In specimens containing mycobacteria survival ranged from 1 to 100%, depending upon the species of mycobacterium. We hasten to add that laboratory experiments were carried out on strains of mycobacteria from stock cultures in nutrient-free distilled water and that these organisms may not accurately reflect the survival characteristics of mycobacteria recovered from the environment.

Survival of mycobacterial cells could probably be increased with a reduction in contact time with the detergent or by the use of lower concentrations of detergent. Stottmeier and coworkers demonstrated that *P. aeruginosa* and *Proteus vulgaris,* as well as gram-positive species, were destroved in concentrations of detergent as low as 0.004% during a 5-h contact time. *Mycobacterium tuberculosis* was found to be resistant (11).

Our method will both simplify the decontamination of large amounts of water and increase the recovery and quantitation of water-borne mycobacteria.

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