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Use of Cetylpyridinium Chloride and Sodium Chloride for the Decontamination of Sputum Specimens That Are Transported to the Laboratory for the Isolation of *Mycobacterium tuberculosis*

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A method is presented for the decontamination, liquefaction, and concentration of sputum specimens that are in transport more than 24 h. The method is inexpensive, and culture results compare well with those obtained with the accepted *N*-acetyl-L-cysteine and sodium hydroxide method for the isolation of tubercle bacilli. The working solution, 1% cetylpyridinium chloride and 2% sodium chloride, is mixed in equal volumes with sputum before the specimens are shipped. Tubercle bacilli remained viable after 8 days of exposure to this solution. Only Lowenstein-Jensen medium was used because the cetylpyridinium chloride in the inoculum remains active on 7H10 or other agar base media and partially inhibits the growth of tubercle bacilli.

In mycobacteriology, there continues to be a universal need for an effective, inexpensive decontaminating substance for sputum specimens that are in transport more than 24 h. The purpose of this report is to show that the treatment of sputum specimens with a solution of sodium chloride and cetylpyridinium chloride (CPC) effectively liquefies and decontaminates the specimen, yet the tubercle bacilli remain viable for at least 8 days. Therefore, the procedure described here may prove useful when specimens cannot be processed the same day of collection.

MATERIALS AND METHODS

The working solution contains 1% CPC (wt/vol) and 2% sodium chloride (wt/vol) (CPC-NaCl) in distilled water. This solution is self-sterilizing and should remain stable if kept tightly capped and away from excessive heat and light. Crystals that formed in the working solution or in specimens with CPC-NaCl that were below 22 C were dissolved by gently warming these solutions before use or further processing.

The sputum specimens were put into 50-ml screw-cap centrifuge tubes, and an equal volume of the CPC-NaCl working solution was added to each specimen. The final concentrations of CPC and sodium chloride were then 0.5% and 1%, respectively. The tubes were capped and shaken until the specimens appeared liquid. After being shipped or a designated decontamination period, the tubes were filled to near the top with sterile distilled water, capped, and then centrifuged at near 2,000 × *g*. The liquid was decanted, and the sediment in each tube was resuspended in 1 to 2 ml of sterile distilled water, physiological saline, or 0.2% bovine albumin fraction V. The resuspended sediment was inoculated on slants of Lowenstein-Jensen medium (L-J) or modified L-J. All cultures were incubated at 35 to 37 C. The modified L-J had 40 U of sodium penicillin added per ml before inspissation.

The *N*-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) decontamination procedure (2) or a modification of it was used as a control procedure. In the modified NALC-NaOH procedure, the concentration of NaOH was increased from 1 to 2% in the specimen during decontamination.

RESULTS

Preliminary investigations demonstrated that the relative number of viable tubercle bacilli was not reduced after 8 days of exposure to CPC at room temperature.

In other experiments, specimens were divided into approximately equal amounts. One part was treated with CPC-NaCl for 5 days before it was concentrated and inoculated on L-J slants. The other part was processed immediately by the NALC-NaOH procedure and inoculated on L-J slants. The results showed (Table 1) that CPC might be as good a decontaminant for the isolation of tubercle bacilli as the NALC-NaOH method and that further studies would be valuable.

The Tuberculosis and Parasitology Laboratory processes a large number of specimens that are received by mail from satellite laboratories. As shown in Table 2, the tubercle bacilli were isolated at approximately the same frequency by both methods. Furthermore, other mycobacteria were isolated more often, and the contamination rate was lower when the specimens were treated by the CPC-NaCl method. Of the mycobacteria cultures other than *Mycobacterium tuberculosis* that were isolated by the CPC-NaCl method, 25 were *M. avium-intracellulare* and 2 were *M. scrofulaceum*.

DISCUSSION

The quaternary ammonium, surface active compound CPC or 1-hexadecyl pyridinium chloride is used as a topical antiseptic (3), and its antiseptic action is similar to cetylpyridinium bromide. Both of these compounds are inexpensive and have been proposed as decontaminants of sputum specimens to isolate tubercle bacilli. Stottmeier and Woodley (10th International Congress for Microbiology, Mexico City, August, 1970) reported using CPC with dithiothreitol, a mycolytic substance. They attempted to develop an alternate procedure for the NALC-NaOH procedure for the processing of sputum specimens. They did not find a concentration that was low enough to not inhibit mycobacterial growth on 7H10 agar medium and yet high enough to continue to give satisfactory decontamination. Boulahbal and Grosset (1) found that cetylpyridinium bromide was toxic to tubercle bacilli.

In this investigation sputum was liquified and decontaminated with a solution of 1% CPC and 2% sodium chloride that was mixed in equal volumes with the sputum. These results compared well with the NALC-NaOH procedures that were used as controls. In one part of the investigation, 1,602 specimens were divided and mailed with one part mixed with CPC-NaCl and, although the sodium hydroxide concentration had been increased to decontaminate the other part, the CPC-NaCl method had a lower contamination rate (3.8% versus 5.7%) and a larger number of mycobacterial cultures isolated (68 *M. tuberculosis* and 31 other versus 66 *M. tuberculosis* and 17 other). Also, tubercle bacilli in the sputum resisted the bactericidal action of the 0.5% final concentration of CPC for 8 days.

The CPC-NaCl method is not intended to replace the NALC-NaOH method for processing fresh specimens because it requires a longer time for decontamination and the residual CPC partially inhibits mycobacterial growth on 7H10 agar medium. But these results indicate that CPC-NaCl may be considered as an inexpensive and effective alternate decontaminant for the isolation of tubercle bacilli from sputum specimens that must be in transport for more than 24 h.

TABLE 1. *Culture results on the initial 34 sputum specimens used to compare CPC-NaCl and NALC-NaOH decontamination procedures*[a]

NALC-NaOH	CPC-NaCl		
	+	−	Contaminated
+	11	1	0
−	1	20	1
Contaminated	0	0	0

[a] One-half of the specimen was processed immediately by the NALC-NaOH procedure; the other half was treated with CPC-NaCl for five days. All positive cultures were *M. tuberculosis*. +, Positive culture; −, negative culture.

TABLE 2. *Culture results on 1,602 sputum specimens used to compare CPC-NaCl and NALC-NaOH decontamination procedures*[a]

Procedure	CPC-NaCl				Total
NALC-NaOH	*M. tuberculosis*	Other mycobacteria	Negative	Contaminated	
M. tuberculosis	54	0	9	3	66
Other mycobacteria	0	6	9	2	17
Negative	14	22	1,342	50	1,428
Contaminated	0	3	82	6	91
Total	68	31	1,442	61	1,602

[a] Specimens were divided, one part had CPC-NaCl added before mailing, and the other part was decontaminated by the modified NALC-NaOH procedure after arrival. The rest of the processing for the two parts of the specimens was essentially the same.

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