Comparison of Sodium Carbonate, Cetyl-Pyridinium Chloride, and Sodium Borate for Preservation of Sputa for Culture of *Mycobacterium tuberculosis*

Antimicrobial susceptibility testing of *Mycobacterium tuberculosis* is essential for a successful treatment of patients, mainly those with a previous history of antituberculosis therapy. Susceptibility testing of strains requires their isolation from sputum samples within 24 to 48 h of collection and storage at 2° to 4°C. If the sample is left at room temperature or in refrigeration for longer periods of time, the recovery of *M. tuberculosis* decreases to 63% and contamination rises to 18% (3). Both problems occur when the laboratory is located far from the patient's home, when refrigeration is not available, or when transportation to the laboratory is inadequate, all common situations in developing countries. Despite these problems, no preservatives are presently used to improve the rate of isolation of *M. tuberculosis* from sputum. In previous studies, sodium carbonate (SC), cetyl-pyridinium chloride (CP), and sodium borate (SB) proved to be good preservatives of this bacterium (1, 5, 6–8).

We compared the efficacy of these three compounds in preserving the viability of *M. tuberculosis* in 58 sputum samples positive for acid-fast bacilli (AFB) from 23 patients with pulmonary tuberculosis who lived in a rural area of Mexico. The study was approved by the Institutional Review Board of our institution.

All samples were initially processed in the local laboratory, in Huauchinango Puebla. Each sample was divided into four equal aliquots and placed in a 50-ml conical test tube with one of the following: (i) SC, 75 mg (J. T. Baker, Xalostoc, Mexico); (ii) 5% SB, 800 µl (J. T. Baker); (iii) 1% CP, equal volume (Sigma Aldrich Chemical Co., St. Louis, Mo.); and (iv) no chemical (control). Aliquots were left at room temperature (25 to 35°C) for 5 to 18 days; 8 samples were stored for 5 days, 17 for 6 days, 13 for 7 days, 6 for 8 days, 8 for 9 days, 1 for 15 days, 3 for 16 days, and 2 for 18 days. Aliquots were then sent to our laboratory where they were digested and were decontaminated with 0.5% *N*-acetyl-cysteine and 2% NaOH (4). Part of the sediment was smeared and was stained first with auramine-

rhodamine (AR) (Sigma-Aldrich) and was then stained with Ziehl-Neelsen (ZN) (Sigma-Aldrich) to confirm results. The remaining sediment was resuspended in Na₂HPO₄-KH₂PO₄ (0.067 M); 0.5 ml was inoculated in Lowenstein-Jensen (LJ) medium (Becton Dickinson, Mexico City, Mexico) and another 0.5 ml in mycobacterial growth indicator tube (MGIT) medium (Becton Dickinson, Sparks, Md.). The remaining sediment was kept at 4°C for 15 days and was redigested if the first culture became contaminated. LJ was incubated at 37°C in 7.5% CO₂ and was examined weekly for 8 weeks. MGIT was incubated in the MGIT 960 instrument (Becton Dickinson). *M. tuberculosis* was identified in positive cultures by DNA probe (Gene Probe, San Diego, Calif.) (2).

AR and ZN stains were AFB positive in 91.4% of smears from samples preserved with SC, 96.6% with SB, and 98.3% of the controls. In contrast, of these samples preserved with CP only, 31% were AR positive and 37.9% were ZN positive.

M. tuberculosis was isolated from at least one of the four aliquots in all 58 sputum samples. AFB were cultured in LJ and MGIT from all samples preserved with SC; however, due to contamination with bacteria and fungi, *M. tuberculosis* was identified in only 86 and 98% of samples, respectively (Table 1). From samples preserved in CP, 98% grew *M. tuberculosis* when cultured in LJ, and in contrast, only 71% grew *M. tuberculosis* when cultured in MGIT. The recovery of *M. tuberculosis* was significantly lower in samples preserved with SB and in controls, mainly due to contamination (Table 1). We observed viability of *M. tuberculosis* in samples preserved with all compounds for 5 to 18 days.

Although this study did not include a systematic analysis of the maximum effective storage time of sputum with the different compounds, we can conclude that the recovery and staining of *M. tuberculosis* were best when sputum was preserved in SC and was cultured in liquid media. Similar yields were obtained when sputum was preserved in CP and cultured in LJ.

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TABLE 1. Comparison of three compounds for recovery of *M. tuberculosis* from 58 sputum samples

Medium used for isolation of <i>M. tuberculosis</i>	No. (%) of samples that yielded <i>M. tuberculosis</i> in different compounds			
	SC	CP	SB	Control ^a
LJ	50 (86)	57 (98) ^b	37 (64)	28 (48)
MGIT	57 (98) ^c	41 (71) ^d	50 (86)	45 (78)
MGIT and LJ	49 (84)	41 (71)	34 (59)	24 (41)
Total	58	57	53	49

^a All comparisons between controls and the three compounds, except for CP in MGIT, showed statistical significance (χ^2 ; $P < 0.05$) in their efficacy in recovery of *M. tuberculosis*. Control contains no preservative.

^b Comparison between CP and SB showed statistical significance (χ^2 ; $P = 0.015$).

^c Comparison of SC with the other compounds showed statistical significance (χ^2 ; $P < 0.02$).

^d Comparison between LJ and MGIT in presence of CP showed statistical significance (χ^2 ; $P < 0.02$) in recovery of *M. tuberculosis*.

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M. Bobadilla-del-Valle

A. Ponce-de-León

M. Kato-Maeda

A. Hernández-Cruz

J. J. Calva-Mercado

B. Chávez-Mazari

B. A. Caballero-Rivera

J. C. Nolasco-García

J. Sifuentes-Osornio*

*Department of Infectious Diseases
Instituto Nacional de Ciencias Médicas
y Nutrición Salvador Zubirán
Mexico City, Mexico*

*Phone: (52-55) 73 12 00, ext. 2174

Fax: (52-55) 13 39 45

E-mail: jso@quetzal.innsz.mx