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Isolation of tubercle bacilli from sputum samples of patients in the field studies by the cetylpyridinium chloride-sodium chloride & sodium hydroxide methods

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A total of 125 sputum specimens, collected in the field, were homogenised, aliquoted in sterile universal containers and randomly allocated to the cetylpyridinium chloride - sodium chloride (CPC-NaCl) method and sodium hydroxide (NaOH) method for culture of tubercle bacilli. After storage for 8 days at ambient temperature in the field laboratory at Thiruvallur, the aliquots were transported to the main laboratory at Madras where they were processed for culture by the respective methods. The yield of positive cultures in the CPC-NaCl (31/125) method was only marginally better than that in the NaOH method (27/125) (95% Cl being-3.4 to 9.8%), while the contamination of cultures was significantly less in the CPC-NaCl method (3/125) than in the NaOH method (12/125) (95% Cl being 2.2 to 12.2%). As the CPC-NaCl method has advantages over the NaOH method in reducing contamination, in augmenting the yield of positive cultures and also in its simplicity, it can be applied in field studies.

Key words Cetylpyridinium chloride-sodium chloride method-field study - *Mycobacterium tuberculosis* - NaOH method-storage of sputum

It is known that storage of sputum samples at ambient temperature beyond 3 days and then processing for culture of tubercle bacilli by the sodium hydroxide (NaOH) method results in significant loss of positive cultures'. A simple and inexpensive procedure, recommended to preserve the viability of tubercle bacilli in sputum samples during transit up to 8 days, is the addition of an equal volume of cetylpyridinium chloride (1%) - sodium chloride (2%) (CPC-NaCl) solution[2]. CPC, a quaternary ammonium compound, at a final concentration of 0.5 per cent did not kill tubercle bacilli when exposed for 8 days[3,4]. This procedure was found to be comparable to the N-acetyl-L cysteine sodium hydroxide (NALC-NaOH) method[3]. Recently, in a controlled laboratory experiment where a comparison was made in fresh and stored samples, the proportion of positive cultures obtained by CPC-NaCl method was significantly higher than in the NaOH method when sputum samples were stored at ambient temperature and processed on the 7th day[5]. The aim of the present study was to compare the culture results of aliquots of the same sputum specimens, collected, stored in the field with and without CPC reagent at ambient temperature and processed on the 8th day for culture of tubercle bacilli by the CPC-NaCl and NaOH methods, respectively.

Material & Methods

Patients, sputum specimens and conditions for storage : The sputum samples were collected from 2 groups of patients. The first group comprised patients who were newly diagnosed based on bacteriological examinations and the second group included bacteriologically proven positive patients undergoing regular treatment. From the latter group (about 60%) sputum specimens were collected during their follow up (mean average 2 1/2 months). The period of study was from October 1993 to May 1994. The sputum specimens were collected from the patients in sterile universal containers at their residence in the respective hamlets or villages (situated on an average of about 20 km radius from the field laboratory) and brought to the laboratory at Thiruvallur, 40 km away from the main laboratory at Madras. Each specimen was homogenised by adding 2 to 3 sterile glass beads and shaken for one minute and aliquoted in equal quantities in bottles and were randomly allocated to the CPC-NaCl method[3] and NaOH method[6]. The aliquots allocated to the CPC-NaCl method were thoroughly mixed immediately with an equal volume of CPC-NaCl reagent. All the aliquots, after storage for 8 days at ambient temperature (30°C on an average) were transported to the main laboratory and processed on the same day for culture by the respective methods by two different technicians.

The centrifuged deposit of the CPC-NaCl treated sample was diluted with about 20 ml of sterile distilled water, shaken throughly and centrifuged again. A loopful of the deposit was inoculated onto 2 Lowenstein-Jensen (LJ) slopes.

In both the methods, the LJ slopes were incubated for 8 wk with weekly examination for growth of *Mycobacterium tuberculosis* and the cultures were checked by a senior microbiologist who was unaware of the treatment procedures of the samples.

Statistical methods used for data analysis: The number of positive cultures as well as the number of contaminated cultures in CPC-NaCl and NaOH methods were determined and the proportion (%) of these cultures calculated. The proportion was tested for significance of difference using McNemar's chi square and 95 per cent confidence interval for the difference in proportions calculated.

Results & Discussion

The culture results obtained in the CPC-NaCl method and NaOH method, for 125 sputum specimens are presented in the Table. The yield of positive cultures in the CPC-NaCl method (CPC added on the same day of collection) and in the NaOH method (NaOH added after storage for 8 days and processed immediately) were 31 and 27 respectively, but the difference was not statistically significant (95% Cl being -3.4 to 9.8%). Of the 125 specimens, 27 were smear positive by the direct smear examination. Of the smear positive specimens, 74 and 70 per cent were culture positive by the CPC-NaCl and NaOH methods, respectively. The rate of growth of tubercle bacilli was similar in NaOH and CPC-NaCl methods (data not presented). The proportion of contaminated cultures in the CPC-NaCl method (3/125) was less than that observed in the NaOH method (12/125) and the difference was statistically significant (95% Cl being 2.2 to 12.2%). In the previous study[5] also the proportion of cultures contaminated by the CPC-NaCl method (111220) was significantly less than in the NaOH method (42/220) (95% Cl being 8.1 to 20.0%). Similarly, Smithwick *et al*[3], in their uncontrolled study with 1602 sputum specimens, recorded 5.6 per cent contamination in NALC-NaOH method compared to 3.8 per cent in CPC-NaCl method.

It is evident that the CPC-NaCl method has advantages over the standard NaOH method in reducing

Table. Culture results of 125 sputum specimens processed for the isolation of tubercle bacilli by the CPC-NaCI and NaOH methods

		CPC-NaCl method						
	Growth*	3+	2+	1+	Col.	Neg.	Cont.	Total
NaOH, method	3+	-	-	-	-	-	-	0
	2+	1	5	2	-	2	1	11
	1 +	-	4	-	l	-	-	5
	Col.	-	-	1	6	4	-	11
	Neg.	-	-	4	5	77	-	86
	Cont.	-	1	-	1	8	2	12
	Total	1	10	7	13	91	3	125

*3+ confluent growth; 2+, innumerable number of colonies: 1+, > 20 but < 100 colonies; col., 1 to 19 colonies; neg, culture negative; Cont, culture contaminated

the contamination and in yielding more positive cultures when the sputum specimens require storage even up to 8 days. In addition, the CPC treated sputum specimens can be centrifuged and processed at a suitable and convenient time by the laboratory personnel and the possibility of exposing sputum to decontaminating agents such as NaOH or oxalic acid for longer period than necessary can be avoided while the technicians attend to other work simultaneously.

The observations of this preliminary study warrant a large scale investigation on the storage of unhomogenised sputum specimens with CPC-NaCI reagent under field conditions before they are processed for culture of tubercle bacilli and a comparison of the culture results between the CPC-NaCl and the NaOH methods.

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