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USE OF CETYLPYRIDINIUM CHLORIDE FOR STORAGE OF SPUTUM SPECIMENS AND ISOLATION OF *M. TUBERCULOSIS*

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Summary. **Of 220 sputum specimens collected from pulmonary tuberculosis patients, 85 were culture positive when the sputum aliquots were stored with cetypyridinium chloride (CPC) and processed on** 7th **day (CPC method), whereas only 70 were culture positive when the aliquots of the same specimens were stored without CPC and processed by sodium hydroxide (NaOH) method. The difference in the culture positivity was statistically significant. The number of positive, cultures obtained by the CPC method (85) was comparable to that obtained by the NaOH method before storage (95) and the difference was not statistically significant.**

Introduction

The sputum specimens of chronic pulmonary tuberculosis patients who do not respond to treatment are usually sent to central mycobacteriology laboratories for culture and drug susceptibility tests. Since such laboratories are not many in this country, there could be delay between the collection of the specimen and its processing. It was shown earlier that storage of sputum specimens at room temperature, beyond 72 hours, would significantly reduce the isolation rate of *M. tuberculosis.*[1] So, a storage procedure for sputum specimens that would give better recovery of *M. tuberculosis* will be greatly useful. The present study was undertaken to assess the usefulness of cetylpyridinium chloride (CPC) for storage of sputum specimens at room temperature up to 7 days.

Material and Methods

Sputum specimens of sufficient quantity, when available, were selected for the study. A total of 220 sputum specimens collected from pulmonary tuberculosis patients were studied. Each specimen was homogenised using sterile glass beads and was divided into 3 aliquots of 4-5 ml each. One of these aliquots was taken up for routine culture by the NaOH method,[2] on the same day. The remaining 2 aliquots were randomly allocated to NaOH and CPC methods. To the sputum aliquot allocated to the CPC method, an equal amount of CPC(1%)-NaCl (2%) reagent was added and after shaking well, it was kept along with the other aliquot in a cupboard at ambient temperature. On the 7th day, these two aliquots were processed for culture by the respective methods.

CPC method : The procedure described by Smithwick *et al*[3] was followed. In brief, the sputum aliquot, treated with CPC-NaCl reagent, was centrifuged at 3000 rpm for 15 minutes and after decanting the supernatant, the deposit was suspended in 1.8 ml of sterile distilled water. A loopful of this suspension was inoculated onto each of 2 Lowenstein Jensen (LJ) medium slopes. Cultures were examined every week up to the 8th week and any growth was recorded as described.[2]

Results

Comparison of the culture results obtained on 220 sputum specimens processed by the CPC and NaOH methods after storage, with that obtained by the NaOH method before storage is presented in Table 1. The numbers of culture positives before and after storage by the NaOH method were 95 and 70 and the difference was highly significant (McNemar test : $P < 0.001$). The number of culture positives obtained by the CPC method after storage (85) was comparable to that obtained by NaOH method (95) before storage and the difference was not statistically significant ($P = 0.08$).

Of the 83 specimens which showed more than 19

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Table 1. *Comparison of culture results by NaOH and CPC methods after storage of sputum specimens with those obtained by NaOH method before storage*

Before storage	After storage												
	NaOH						CPC						Total
NaOH	3+	2+	1+	Col.	Neg.	Cont.	3+	2+	1+	Col.	Neg.	Cont.	
3+*	4	12	4	-	-	6	11	12	-	-	-	3	26
2+	1	13	7	9	7	7	9	16	11	4	3	1	44
1+		1	1	4	4	3		3	3	4	2	1	13
Col.	-	-	1	4	6	1	-	1	-	3	7	1	12
Neg.	-	2	-	2	89	22	-	1	-	1	109	4	115
Cont.	-	3	2	-	2	3	2	2	1	1	3	1	10
Total	5	31	15	19	108	42	22	35	15	13	124	11	220

*3 + = confluent growth; 2 + = innumerable colonies; 1 + = more than 20 but less than 100 colonies; Col. = less than 20 colonies; Neg. = culture negative; Cont. = culture contaminated.

Table 2. *Culture results according to NaOH and CPC methods after storage*

		CPC method						Total
	Culture grades	3+	2+	1+	Col.	Neg.	Cont.	
NaOH method	3+*	3	2					5
	2+	13	14	1		2	1	31
	1+	1	9	3	1		1	15
	Col.	1		5	8	5		19
	Neg.	1	2	3	2	93	7	108
	Cont.	3	8	3	2	24	2	42
	Total	22	35	15	13	124	11	220

*as indicated in Table 1.

colonies by the NaOH method before storage, 73 (88%) and 56 (67%) had positive culture by the CPC and NaOH methods respectively after storage. Among 12 specimens which showed less than 20 colonies before storage, 4 and 5 were positive for culture by the CPC and NaOH methods respectively after storage

The culture results by the CPC and NaOH methods after storage are presented in Table 2. Of the 70 specimens which were positive by the NaOH method, 7 were negative and 2 were contaminated by the CPC method, whereas of 85 specimens which were positive by the CPC method, 8 were negative and 16 were contaminated by the NaOH method. Of the 42 specimens which were contaminated by the NaOH method, 16 were positive by the CPC method whereas of the 11 specimens contaminated by the CPC method, only 2 were positive by the NaOH method.

Discussion

The use of CPC for decontamination of sputum specimens in transit in temperate countries was reported by Smithwick *et al*[3] and Tazir *et al.*[4] In the

former investigation on 1602 specimens, which were transported from satellite centres to the central laboratory, the yields of positive cultures by the CPC and N-acetyl L-cystein-Sodium hydroxide (NALC-NaOH) methods were comparable (68 and 66 cultures respectively). In the present study, the CPC method was compared with the NaOH method which is generally followed in most of the referral laboratories in India. Using NaOH method, Paramasivan *et al*[1] had shown that the culture positivity decreased from 88% before storage to 65% after storage for 7 days. Similarly, in the present study, the number of culture positives decreased from 95 before storage to 70 after storage by NaOH method. By storing the sputum specimens with CPC reagent, the reduction in the culture positivity has been largely offset. This is evident from the observation that 85 of 220 sputum specimens were positive for culture by the CPC method compared to 70 by the NaOH method (P = 0.01). After storage, the culture positives obtained by the CPC method in the present study were comparable to the results obtained by NaOH method before storage. Similarly, Smithwick *et al*[3] had shown, in a pilot study, that the culture positivity in the CPC method after storage was comparable to the results obtained by NALC-NaOH method before storage.

The advantages of the CPC method are that the reagent is stable at room temperature, easy to prepare, inexpensive and also self sterilizing. More over, the specimens need only a single centrifugation before inoculation whereas the NaOH method requires careful processing of the specimen in different steps which are time bound.

The results suggest that the yield of culture positives would be higher with the CPC method than with the NaOH method when sputum specimens need to be processed after storage at room temperature up to 7 days. Hence, the CPC method may be applied under programme conditions.

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