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Shifting from modified Petroff's to NALC-NaOH method for processing of sputum specimens for solid culture

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Abstract

Sputum decontamination by modified Petroff's method is routinely used by many laboratories for isolation of mycobacteria in solid culture. With the introduction of line probe assay (LPA) for MDR TB diagnosis, our laboratory adopted the recommended NALC–NaOH decontamination procedure for sputum processing. Out of 284 and 1039 specimens processed by modified Petroff's and NALC-NaOH, significantly high percentage (P=0.004) of contamination was observed by the latter method. In the initial period, the contamination reached 19.5% with the NALC-NaOH method and reduced with time. Laboratories adopting new decontamination methods may be careful during the initial months for avoiding the loss of specimen due to contamination while adopting LPA.

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Introduction

Tuberculosis remains to be one of the leading causes of death in the world today and 80% active cases are found in 22 low and middle income countries [1]. In programmatic condition the diagnosis still relies on microscopical observations of smear, a method having low sensitivity with paucibacillary specimens [2]. Due to the emergence of multi drug resistant (MDR) and extensively drug resistant (XDR) Tuberculosis (TB), countries are scaling up with establishment of culture and drug susceptibility testing laboratories and also adopting newer molecular based techniques like line probe assay (LPA) for its early detection. For processing of sputum specimen, mostly contaminated with oral bacteria and environmental fungi, modified Petroff's method with 4% NaOH is being used by many laboratories attempting to isolate mycobacteria. The introduction of new technology like LPA also necessitated the shifting of decontamination procedure from modified Petroff's to adoption of N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method for decontamination. During the transitional phase of adoption of newer decontamination method, that may result in higher contamination rates for culture. Hence laboratories may have to develop a strategy to overcome the transition for getting a good yield of *Mycobacterium tuberculosis* in culture.

Material and Methods

This study was conducted in RMRC, Bhubaneswar from January to September 2014. Sputum specimens were collected from suspected pulmonary TB patients those who were attending outpatient department of Capital Hospital, Bhubaneswar. Samples were transported to RMRC, Bhubaneswar after the Laboratory Technician of Capital hospital made slides for Acid Fast Bacilli (AFB) microscopy by Ziehl-Neelsen (ZN) staining. Written informed consent was obtained from all patients who have participated in this study. The sputum specimens were processed at National Reference Laboratory of Regional Medical Research Centre, Bhubaneswar for microscopy and isolation of *M. tuberculosis*. Duplicate smears were made and stained by both ZN and auramin staining methods and graded following International Union of Tuberculosis and Lung Diseases (IUTLD) guidelines.

Sputum specimens were processed by modified Petroff's method up to May 2014 after which processed by NALC-NaOH method [3] due to introduction of Line Probe Assay for detection of MDR TB in the laboratory. In brief, for modified Petroff's method, 3-5 ml of sputum was homogenized for 15 minutes in a shaker using an equal volume of 4% sodium hydroxide. After centrifugation at 3,000 rpm for 15 min, the deposit was neutralized with 45 ml of sterile distilled water.

The samples were again centrifuged and from the sediment, a loop full was inoculated in to two slants of Lowenstein-Jensen (LJ) and to one slant of LJ containing para-nitro benzoic acid (PNB). For NALC-NaOH method, 3-5 ml of sputum sample, an equal volume of NALC-NaOH citrate reagent was added and the tube was vortexed briefly. Following 15 min of incubation at room temperature, the volume was made to 50 ml with 0.067 M phosphate buffer (pH 6.8) and the contents were mixed by inversion. Bacteria were sedimented by centrifugation at 3,000 x for 15 min. The supernatant was discarded after centrifugation at 3,000 x g for 15 min, and the pellet was resuspended in 1 ml of phosphate buffer. From the pellet a loop full was inoculated like the modified Petroff's method. The culture slants were incubated at 37°C. All slopes slants were observed for occurrence of growth daily for first week and then at weekly intervals for 8 weeks. The isolates were identified by following tests: rate of growth, optimum temperature of growth, colony morphology, pigmentation, growth in PNB, catalase test and niacin test which confirmed that all isolates were *M.tuberculosis*. The Ethical Committee of RMRC, Bhubaneswar approved the study protocol.

Results

Out of 40 and 179 positive smear specimens processed by modified Petroff's and NALC-NaOH method, isolation of *M. tuberculosis* was successful in 82.5 and 86.0 percent specimens respectively (Table 1). Out of 244 and 860 negative smear specimens, 7.0 and 8.4 percent specimens were found positive respectively by modified Petroff's and NALC-NaOH method (Table 1). The rate of contamination between the two methods was similar in positive smear specimens. While a significant proportion of negative smear specimens were found contaminated with NALC-NaOH method (P=0.001).

The overall contamination rate observed was also significantly higher (P=0.004) in NALC-NaOH method (8.3% vs 3.5%). The month wise contamination rate was between 0 and 8.5 percent with modified Petroff's method, while contamination increased up to 19.5% in the initial month with NALC-NaOH method (Table 2).

Culture result with modified Petroff's					Culture result with NALC-NaOH			
Smear Grade	No. tested	Positive (%)	Negative (%)	Contamination (%)	No. tested	Positive (%)	Negative (%)	Contamination (%)
3+	12	11 (91.7)	0	1 (8.3)	52	48 (92.3)	0	3 (5.8)
2+	8	7 (87.5)	0	1 (12.5)	52	46 (88.5)	1 (1.9)	5 (9.6)
1+	17	13 (76.5)	3 (17.7)	1 (5.9)	57	46 (80.7)	7 (12.3)	4 (7.0)
Scanty	3	2 (66.7)	1 (33.3)	0	18	14 (77.8)	4 (22.2)	0
Neg	244	17 (7.0)	218 (89.3)	7 (2.9)	860	72 (8.37)	706 (82.1)	74 (8.6)
Total	284	50 (17.6)	222 (78.2)	10 (3.52)	1039	226 (21.8)	718 (69.1)	86 (8.3)

Table 1: Isolation of *M. tuberculosis* from sputum samples following different methods of decontamination

Month	Number of specimens processed by Petroff's	Contamination(n)%	Number of specimens processed by NALC-NaOH	Contamination(n)%
January	64	2 (3.1)	ND	-
February	82	7 (8.5)	ND	-
March	54	1 (1.9)	ND	-
April	84	0	ND	-
May	ND	-	169	33(19.5)
June	ND	-	260	22 (8.5)
July	ND	-	247	12 (4.9)
August	ND	-	363	19 (5.2)

Table 2: Sputum processing month wise for culture on LJ

ND: Not done LJ: Lowenstein-Jensen

Discussion

Sputum specimens collected from patients were mainly contaminated with other microbes. Decontamination with 4% sodium hydroxide may be detrimental to mycobacteria to some extent and the percentage of organisms killed varies according to the method used. It was reported that NALC coupled with 2% sodium hydroxide provided fairly reasonable recovery of mycobacteria compared to 4% sodium hydroxide. During culture one of the major concerns is to minimize contamination to save the loss of specimen. As the Petroff's method uses more concentration of NaOH which kills contaminating organisms more effectively, many laboratories prefer it for solid culture. For detection of MDR TB many laboratories are scaling up and newer technologies are being employed for rapid diagnosis. Due to introduction of LPA and liquid culture Mycobacterial Growth Indicator Tube (MGIT) laboratories adopt the recommended NALC-NaOH decontamination procedure which uses lesser percentage of sodium hydroxide. With the introduction of the new decontamination method like NALC-NaOH, contamination of 19.5% was reported resulting in loss of specimens. It may be wise to process a portion of the specimen by modified Petroff's method and another portion by NALC-NaOH initially for a month or two till the contamination rate stabilizes. The study reported here did not compare among the decontamination methods, but recommend laboratories to develop a strategy to overcome the loss of specimen during changing of decontamination methods with the introduction of LPA to their laboratory.

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Conflict of Interest

None to declare

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