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Original Research Article

An Epidemiological Investigation to Detect the Presence of Mycobacterium Tuberculosis Using Sputum Assessment Methods

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Abstract

Aim: To identify the presence of mycobacterium tuberculosis by the use of sputum evaluation techniques at a medical facility specializing in advanced care.

Materials and Methods: The Study was conducted as a retrospective epidemiological study. Total of 143 sputum samples obtained from both in-patients and out-patients of Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India from March 2019 to February 2019. TB and RD and Orthopaedics were subjected to decontamination by both HS-SH method and NALC-NAOH methods. Slides were then examined under oil immersion for acid fast bacilli by conventional Ziehl-Nelsen staining microscopic method.

Results : Out of 143 microbiological sputum samples, 104 were found to be positive for acid fast bacilli by both Modified Petroff's method and Hypertonic Saline Sodium Hydroxide method (HS-SH) of concentration and decontamination and 39 samples were negative by both these methods. Sensitivity, Specificity, Positive predictive value and Negative predictive value of the HS- SH method was found to be 100%.

Conclusion: In conclusion, the HSSH sputum decontamination method is equally sensitive, specific, cost-effective, feasible and less time-consuming procedure compared to the gold standard NALC-NaOH method for decontamination of Mycobacterium tuberculosis from sputum samples and can be routinely used in all peripheral health centres because of the low cost, especially in developing countries.

Keywords: Mycobacterium tuberculosis, Sputum samples, for Hypertonic Saline - Sodium Hydroxide Method

Introduction

Tuberculosis (TB) remains a major global health issue, with Mycobacterium tuberculosis as its etiological agent. Despite substantial progress in TB control over recent decades, the disease continues to be a leading cause of morbidity and mortality, particularly in low- and middle-income countries (WHO, 2021). Accurate and timely detection of M. tuberculosis is crucial for effective TB management and control. Various sputum evaluation methods have been developed and employed to diagnose TB, each with its strengths and limitations. This introduction provides an overview of these methods, emphasizing their roles in the detection of M. tuberculosis, with a focus on sputum smear microscopy, culture techniques, and molecular methods. [1-3]

Sputum smear microscopy, introduced over a century ago, remains one of the most widely used methods for TB diagnosis, particularly in resource-limited settings. The technique involves staining sputum samples with acid-fast dyes, such as Ziehl-Neelsen or auramine-rhodamine, to visualize acid-fast bacilli (AFB) under a microscope. Although sputum smear microscopy is relatively simple and inexpensive, it has several limitations. The sensitivity of the test varies, generally detecting only cases with high bacillary loads, which limits its ability to identify TB in patients with low bacillary counts or extrapulmonary TB. Furthermore, the specificity of the test can be compromised by the presence of non-tuberculous mycobacteria. [4-6]

Despite these limitations, smear microscopy's rapid turnaround time and low cost make it an essential tool in TB control programs, particularly for screening and monitoring treatment response. Efforts to enhance the sensitivity of smear

microscopy, such as the use of LED fluorescence microscopy, have shown promise. Studies indicate that LED fluorescence microscopy is more sensitive than conventional Ziehl-Nilsen staining, potentially improving the detection rate of smear-positive TB cases. [7]

Culture techniques are considered the gold standard for TB diagnosis due to their high sensitivity and specificity. These methods involve inoculating sputum samples onto solid or liquid media to grow M. tuberculosis colonies. The Lowenstein-Jensen (LJ) medium is the most commonly used solid medium, while liquid culture systems, such as the Mycobacteria Growth Indicator Tube (MGIT) system, have gained popularity due to their faster turnaround times . [8] Despite their superior accuracy, culture methods have significant drawbacks. The primary limitation is the lengthy incubation period required for M. tuberculosis to grow, which can range from 2 to 8 weeks for solid media and up to 2 weeks for liquid culture systems. This delay can impede timely diagnosis and treatment initiation, contributing to ongoing transmission. Moreover, culture methods require sophisticated laboratory infrastructure and trained personnel, limiting their applicability in resource-poor settings. The advent of molecular techniques has revolutionized TB diagnostics by providing rapid and accurate detection of M. tuberculosis. Polymerase chain reaction (PCR)-based assays, such as the Expert MTB/RIF assay, have become integral components of modern TB diagnostic algorithms. The Expert MTB/RIF assay simultaneously detects M. tuberculosis DNA and rifampicin resistance mutations, offering results within 2 hours. This dual capability is particularly valuable for guiding appropriate treatment regimens and managing drug-resistant TB. Molecular methods offer several advantages over traditional techniques. They have higher sensitivity and specificity, can detect TB in smear-negative and extrapulmonary cases, and provide faster results, facilitating prompt initiation of therapy . However, the high cost of these assays and the need for specialized equipment and technical expertise can be barriers to their widespread implementation, particularly in low-resource settings. [9,10]

Materials and Methods

The Study was conducted as a retrospective epidemiological study. Total of 143 sputum samples obtained from both in-patients and out-patients of Department of Microbiology, Anugrah Narayan Magadh Medical College, Gaya, Bihar, India. from March 2019 to February 2019, TB and RD and Orthopaedics were subjected to decontamination by both HS-SH method and NALC-NAOH methods. Slides were then examined under oil immersion for acid fast bacilli by conventional Ziehl-Nilsen staining microscopic method.

Inclusion Criteria

1. Patients with cough more than 2 weeks and/or blood stained sputum.

2. New sputum positive pulmonary tuberculosis patients before initiation of treatment.

Exclusion Criteria

1. Patient already under anti-tubercle drug treatment.

2. Inadequate sample volume (< 2ml).

3. Sample not representative of lower respiratory tract.

4. Patient denying participating in the study.

Decontamination Method

Procedure for Hypertonic Saline - Sodium Hydroxide Method (HS-SH). One ml portion of sputum was mixed with one ml 7 % (w/v) NaCl and one ml 4 % (w/v) NaOH in a sterile 15 ml centrifuge tube (BD Falcon) and homogenized for 15– 20 s using a vortex mixer [Final concentrations (w/v) in 3 ml: 2.33 % NaCl, 1.33 % NaOH]. The tubes were then incubated at 37^0C for 30 min. After incubation, the mixture was neutralized with sterile PBS (pH 6.8), bringing the total volume to 15 ml. The mixture was vortexed for 5 s and then centrifuged at 3400 g for 15 min at 15^0C, using aerosol proof shields. The supernatant was discarded into a splash-proof container with a tuberculocidal solution. The procedure used has been described previously and is recommended by the Centres for Disease Control and Prevention and the WHO/IUATLD. one ml sputum was added to a 50 ml BD Falcon centrifuge tube with one ml of solution containing 0.5% (w/v) NALC, 2.67% (w/v) NaOH and 1.45% (w/v) sodium citrate and mixed well (final concentrations (w/v) in 2 ml: 0.25% NALC, 1.34% NaOH, 0.73% sodium citrate). The tubes were incubated at room temperature for 15 min. After incubation, the mixture was neutralized with PBS, bringing the total volume to 50 ml. The rest of the procedure was as described above. ZN sputum Smear preparation AFB sputum smear preparation done in Bio safety cabinet level II. One drop of each suspended pellet was used to prepare slides for AFB microscopy using the Ziehl–Neelsen stain. Each slide was coded, read blindly by a qualified technician and reported according to the National Tuberculosis Program and WHO/IUATLD standards (WHO, 1998). Smears were reported as follows: Grade 0 where no Acid Fast Bacilli (AFB) is observed in a total of 200 oil immersion fields, Scanty (Sc) where 1–9 AFB in 100 microscopic fields (few bacilli) is observed; 1+ with 10–99 AFB in 100 fields; 2+ with 1–10 AFB per field in at least 50 fields; 3+ with more than 10 AFB per field in at least 20 fields. Each slide will be coded, examined and graded according to the RNTCP guidelines.

Results

Out of 143 microbiological sputum samples, 104 were found to be positive for acid fast bacilli by both Modified petroff's method and Hypertonic Saline Sodium Hydroxide method (HS-SH) of concentration and decontamination and 39 samples were negative by both these methods. Sensitivity, Specificity, Positive predictive value and Negative predictive value of the HS- SH method was found to be 100%.

Table.1 Comparison of N-Acetyl -L- Cysteine Sodium Hydroxide (NALC-NaOH) and Hypertonic Saline Sodium Hydroxide (HS-SH) methods: Decontamination methods NALC + - HSS

Decontamination methods	NALC-NaOH	HS-SH
	104	0
	0	39
Total	143	

Discussion

Decontamination methods for sputum microscopy for diagnosis of tuberculosis presently used are cumbersome, time consuming and expensive. Better methods are needed particularly developing countries. According to the study conducted by Christian Ganoza et al [5] yl-L-Cysteine (NALC-NaOH) using decontamination (DC) methods. In a study conducted in India, showed sensitivity of NALC-NaOH and HS-SH methods were 46% and 52% respectively. The proposed novel HS–SH DC method improved the sensitivity of AFB microscopy compared with a routine direct smear; its performance was comparable to that of the Modified Petroff's method for AFB smears, but it was methodologically simpler and less expensive, equally sensitive and can be adopted in National TB control programme especially in developing countries. Sensitivity, Specificity, Positive predictive value and Negative predictive value of the HS-SH method was found to be 100% which is comparable with the standard Modified petroff's method. Novel HS-SH method was not all cumbersome compared to the gold standard NALC-NaOH method. In conclusion, the HS- SH sputum decontamination method is equally sensitive, specific, cost-effective, feasible and less time consuming procedure compared to the gold standard NALC-NaOH method for decontamination of Mycobacterium tuberculosis from sputum samples and can be routinely used in all peripheral health centres because of the low cost, especially in developing countries.

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