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Original Research Article

An Epidemiological Study Detecting Mycobacterium Tuberculosis Using Sputum Evaluator Methods: An Observational Study

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Abstract:

Aim: The aim of the present study was to evaluate the detection of mycobacterium tuberculosis using sputum evaluator methods.

Methods: The Study was conducted as a prospective epidemiological study in Department of Microbiology for one year. Total of 100 sputum samples obtained from both in-patients and out-patients of Hospital from various departments like Medicine, Surgery, TB and RD and Orthopedics were subjected to decontamination by both HS-SH method and NALC-NAOH methods.

Results: Out of 100 microbiological sputum samples, 70 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 30 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 caused by Mycobacterium tuberculosis (or Koch's bacillus) even though curable and preventable it is still one of the leading infectious causes of morbidity and mortality especially in developing countries like India. [1] It is estimated that a third of the world's population, is infected with MTB, but most never develop active TB disease. [2] Global incidence of tuberculosis is 9.4 million cases annually, whereas India accounts for 2.0 million i.e., one-fifth of the Global incidence. [3] In India the prevalence rate is 249 per lakh population (2009). The life time risk of a person developing Tuberculosis is 10% (10 out of 100). [4] Diagnosis of Tuberculosis is by a combination of proper history taking, clinical examination, Microbiological evaluation by Sputum microscopy and Culture (Conventional or automated) which is the gold standard and also by correlation with roentgenologic examination. Although a presumptive diagnosis of pulmonary tuberculosis can be made on the basis of patient histories and clinical and radiological findings, the definitive bacteriological diagnosis of tuberculosis continues to depend on the microscopic examination of acid-fast stained sputum smears and then cultural confirmation. Direct microscopy by Ziehl-Neelsen staining to identify acid-fast bacilli (AFB) is the most rapid method, but it lacks sufficient sensitivity and specificity. On the other hand it takes 4 to 8 weeks to culture pathogenic mycobacteria because of their slowly growing nature. Recently there has been great progress in developing rapid, sensitive, and specific tests for the diagnosis of tuberculosis. [5] The only in vivo test available to evaluate M. tuberculosis infection is the tuberculin skin test, which has fair sensitivity but poor specificity. [6]

On the other hand, the new interferon-gamma release assays are specific ex vivo tests. Both methods are based on the measurement of adaptive host immune response. However, none of these tests can accurately distinguish between latent and active TB. [6-8] Other diagnostic tools have been developed for the detection of M. tuberculosis, as well as drug susceptibility and viability, which can be evaluated by metabolic activity responsiveness

(detection of respiration or mRNA synthesis), cell membrane integrity, or nucleic acid detection. [9] Along with these tests, conventional solid and new liquid media-based methods, which can obtain rapid results, have been developed; however, these tests are quite expensive. [10]

In an effort to have a simple, affordable, rapid and safe TB diagnostic system, a simple DNA diagnostic system for MTB detection (herein after referred to as NWU-TB system) was developed, which can be readily applied to some other diseases. This molecular based system aims to possess the high accuracy associated with other molecular based diagnostic systems, but with the low cost structure of SSM. It is envisaged that such a system may replace SSM in SSA.

The aim of the present study was to evaluate the detection of mycobacterium tuberculosis using sputum evaluator methods.

Materials and Methods

The Study was conducted as a prospective epidemiological study in Department of Microbiology, ANM Medical College and Hospital, Gaya, Bihar, India for one year. Total of 100 sputum samples obtained from both in-patients and out-patients of ANM Medical College and Hospital, Gaya, Bihar, India from various departments like Medicine, Surgery, TB and RD and Orthopedics 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-Neelsen 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) [10,11]

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 Centers 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

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

Microbiological sputum samples	N%
Positive	70 (70%)
Negative	30 (30%)

Out of 100 microbiological sputum samples, 70 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 30 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%.

Discussion

Tuberculosis (TB) remains one of the top 10 major public health problems worldwide, with an estimated 10.6 million people falling ill from TB annually. TB caused 1.4 million deaths among HIV-negative people and an additional 187,000 deaths among people living with HIV (PLHIV) in 2021. [12] Accurate, sensitive, and high-quality diagnostic testing, usually performed in centralized laboratories in urban centers, is crucial to improving TB diagnosis and identifying drug resistance. If specimens cannot reach centralized laboratories under conditions that preserve specimen integrity, the performance of downstream tests and their potential impact will be undermined. [13]

Out of 100 microbiological sputum samples, 70 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 30 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%. 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 [14] the sensitivity for acid-fast bacilli (AFB) smears had increased from 28.6% using the direct method to 71.4% Hypertonic Saline Sodium Hydroxide (HS–SH) method and 66.7% N-Acetyl-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. [15]

Several decontamination procedures require that the performance conditions – such as the exposure time to trisodium phosphate – be carefully controlled. Zephiran requires neutralization with lecithin and is not compatible with inoculation on egg-based culture media; the usefulness of chitin solutions for culture has not been evaluated yet; bleach solution is of course unsuitable for cultivation. For the recovery of M. tuberculosis after treatment with CB-18w, a separate protocol with three lytic enzymes, which increases the whole cost, is used in conjunction with CB-18w. However, the initial studies showed an important increase in sensitivity with this method when compared to the NALC-NaOH method. [16,17]

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.

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