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Isoniazid and rifampin drug susceptibility testing: application of 2,3,5-triphenyl tetrazolium chloride assay and microscopic-observation drug-susceptibility assay directly on Ziehl-Neelsen smear positive sputum specimens

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ABSTRACT

The current study was aimed to evaluate the performance of direct 2,3,5-triphenyl tetrazolium chloride assay and direct microscopic observation drug susceptibility assay with indirect Löwenstein-Jensen proportion method directly on Ziehl-Neelsen smear positive sputum specimens.

Methods: Direct acid fast bacilli smear positive sputum specimens ($n = 264$) were subjected to isoniazid and rifampicin drug susceptibility testing by direct 2,3,5-triphenyl tetrazolium chloride assay, direct microscopic observation drug susceptibility assay, and the performances were compared with indirect Löwenstein-Jensen proportion method.

Results: The direct 2,3,5-triphenyl tetrazolium chloride assay demonstrated an overall sensitivity, specificity, positive predictive value, and negative predictive value of 99.2%, 82.4%, 99.2%, and 88.5%, respectively, for the detection of isoniazid and rifampicin resistant Mycobacterium tuberculosis isolates when compared to indirect Löwenstein-Jensen proportion method. Likewise, the overall sensitivity, specificity, positive predictive value and negative predictive value of direct microscopic observation drug susceptibility assay were 98.8%, 82.4%, 99.2%, and 78.2%, respectively.

Conclusion: The direct 2,3,5-triphenyl tetrazolium chloride assay was found to be an economical alternative method for the rapid and accurate detection of isoniazid and rifampicin resistance from direct acid fast bacilli smear positive sputum specimens.

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Introduction

Tuberculosis (TB) continues to be a leading public health problem across the world particularly in developing countries (Southeast Asia accounts for nearly 40% of global TB cases).[1] TB is further complicated by the rising incidence of multidrug resistant (MDR) strains of tubercle bacilli and extensively drug-resistant TB, challenging TB control efforts in several low- and middle-income countries.[2,3] In India, the MDR TB is distributed among 2.9% and 15.3% of newly and previously treated TB cases, respectively, as published by Revised National Tuberculosis Control Program.[4] However, the rising incidence and rate of transmission of MDR and extensive drug-resistant TB may be minimized by timely detection of the cases.[5] Currently available drug susceptibility testing for mycobacterial isolates use an egg or agar based medium. These traditional culture methods detect Mycobacterium tuberculosis (MTB) growth after an average of three weeks under optimal conditions, and the drug susceptibility testing takes additional three to four weeks.[6,7] However, automated systems (broth based) considerably reduced the time to detection of drug resistance of mycobacterial isolates, but require expensive instrumentation and are technically complicated, limiting their application in low resource settings.[8,9] Furthermore, molecular methods used for the characterization of genes that confer resistance to first-line antimicrobial agents such as Isoniazid (INH) and Rifampicin (RIF) are available; however, the high cost of instruments and specialized expertise to perform these methods limit their wide spread use in developing countries.[10] Furthermore, various colorimetric and non-colorimetric assays have been developed recently. However, all these methods were restricted or not evaluated under field conditions, suggesting the prerequisite of an accurate, rapid, inexpensive, and technically simple method for the detection of MDR TB in resource-limited settings.[11–18] In India, most of the mycobacterial clinical laboratories are experiencing intricacy in obtaining drug susceptibility information for MTB isolates due to financial or technical reasons.[19] Moreover, treatment of TB unaware of susceptibility information may increase the risk of treatment failure, the spread of resistant strains, and the development of resistance to additional drugs as well.[20] In view of the above issues, the current study was designed to evaluate the performance of microscopic observation drug susceptibility (MODS) assay and 2,3,5-triphenyl tetrazolium chloride (TTC) assay with indirect proportion method (using Löwenstein-Jensen (LJ) media) directly on Ziehl-Neelsen (ZN) smear positive sputum specimens. Moreover, to our knowledge, this is the first report to investigate the performance of direct TTC assay and direct MODS directly on ZN smear positive sputum specimens.

Materials and methods

Study settings

The study was conducted in the Department of Microbiology, MM Institute of Medical Sciences and Research, Mullana, Ambala, India, a tertiary care hospital (1050 bedded) that also functions as a peripheral center for the Revised National Tuberculosis Control Program.

Patient screening and recruitment

A total of 283 patients, who were positive for direct acid fast bacilli sputum smear microscopy (stained using ZN staining method) and meeting any of the following criteria were recruited: receiving anti tuberculosis treatment, suspected TB treatment failure, or suspected TB relapse cases were included. However, smear negative cases of pulmonary tuberculosis and salivary sputum samples were excluded.

For the present study, a total of two sputum specimens (spot sample at clinic and morning sample at home) from each patient were collected and all the specimens were transported immediately to the microbiology laboratory (Department of Microbiology, M.M.I.M.S.R) and refrigerated (4–8 °C) overnight. Upon receipt of the second sputum specimen, both spot and early morning sputum samples were stained using ZN staining technique and reports were issued to the patients [as all the sputum samples were collected for routine direct Acid fast bacilli (AFB) smear examination]. For this study, the spot sample at clinic and morning sample at home (both direct AFB smear positive sputum samples) were collected and then mixed irrespective of the smear grades obtained in the routine direct AFB smear examination by pouring the sample from one bottle to another containing undrilled glass beads (1–2 mm, Himedia, Mumbai, India). After mixing, the sputum specimens were homogenized (using a Pasteur pipette for one min) and vortexed for two min to assure uniform distribution of bacilli. After homogenization, the sputum specimens were divided into two aliquots. The first aliquot was subjected to direct AFB smear examination and culture on LJ media, direct MODS assay and direct TTC assay. The second aliquot was stored in a deep freezer (REMI; UDFV-185, Mumbai, India) at −80° C for three months or till the drug susceptibility test results were available. If the DST results using the first aliquot of sputum were not interpretable, then the second aliquot was used to repeat the indirect Löwenstein-Jensen proportion method (LJ PM), direct TTC assay and direct MODS assay.

Laboratory methods

Specimen processing and inoculum preparation

The first aliquot of the sputum specimen [2–3 mL in 14 mL BD Falcon™ centrifuge tube (Becton Dickinson, Franklin Lakes, NJ, USA)] was subjected to direct AFB smear examination and N-acetyl L cystiene (NALC)–sodium hydroxide (NaOH) decontamination method (final NaOH concentration 1%).[6] After NALC–NaOH decontamination method, the sediment in each tube was suspended again in phosphate buffered saline to 2 mL and mixed well. This resuspended sediment was used as a common inoculum source for direct TTC assay, direct MODS assay, and indirect LJ proportion method.