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Detection of non-tuberculous mycobacteria from sputum samples

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ABSTRACT

NTM infections are an emerging global public health problem, especially in developing world. NTM and MTBC were proven to be responsible for various lungs, soft and skin tissues, and disseminated infections. Microscopy of AFB is rapid; however it does not discriminate between MTBC and NTM. Biochemical tests are time taking and interpretation of results may be difficult. The GenoType® Mycobacterium CM/AS assay is reliable and rapid detection method in species of mycobacteria, which can give patients with the onset of early targeted therapy. In present study, 189 patients detect by sputum sampling from total 3320 patients.

Keywords: NTM, Sputum, Diagnosis, Clinical, Lucknow.

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INTRODUCTION

Infection of Non tuberculosis Mycobacteria (NTM) is regard as serious health concern around the world[1-4]. Mycobacterium genus belongs to Mycobacteriaceae family includes gram-positive bacteria and one of many mycolic acid involving genera among the Actinomycetes order. In the past, they were reported as mycobacterium rather than tuberculosis, atypical mycobacteria, but later, known as NTM[5-8]. Typical mycobacteria are spreading by cough from infected person to healthy individual in the near surrounding, which usually affect the lungs; NTM which is readily isolated from water and soil among the group of diverse species of *Mycobacteria* present in the atmosphere. Except *Mycobacterium tuberculosis complex* (MTBC) and *Mycobacterium leprae*, NTM incorporate all other species of mycobacteria. NTM are divided into two types of species one is Rapid growing mycobacteria (RGM) and other is Slow growing mycobacteria (SGM). Occurrence of NTM has been reported in human respiratory and non-respiratory diseases. A continuous global rise in NTM incidence is a big concern for physicians and microbiologists. Earlier in various developing countries, the higher occurrence of tuberculosis has surpassed the identification of NTM associated infections[9-12]. Most of these are present mainly in the form of saprophytes in the natural surrounding and have been identified as an etiological agent in human diseases. Now days this trend is changing with great pace. Higher reporting of NTM related pulmonary disease leads to international attention[13-15]. As of, 2014 estimated incidence of respiratory disease was 33–65 per 100,000 due to NTM[16]. In present study, we detected non-tuberculosis mycobacteria by sputum sampling from patients.

MATERIALS AND METHODS

Study Design and Study Setting

This study designed as a prospective hospital based study in Northern India at department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, UP India.

Selection of Subjects

Patients were suspected of tuberculosis cases and fulfilling the criteria for the inclusion of in and out patients in different wards of SGPGIMS, Lucknow was included in the study. All samples are received in the laboratory from all over the U.P, India. Study Performa was include history of current and past illness of tuberculosis, history of anti-tubercular treatment past and history related to other disease.

METHODOLOGY

Collection of Specimens and Laboratory Procedures

Pulmonary specimens were collected at various wards of SGPGIMS and send to the Mycobacteriology laboratory, SGPGIMS, within an hour's of collection of specimens. All the samples were collected by resident In-charge on duty as a part of its diagnosis. Samples were confirmed for leakage and outside of tubes were wiped with spirit swab before processing. All the specimens were processed on the day of

collection. Samples were processed in laminar flow hood with Class II Biological Safety level. There have no separate samples were collected for the research. Data of clinical and epidemiological was collected from patients with hospital information system and patient history case sheets.

Labeling of Samples

– On the specimen container

– On the field data collection form

– On the log book

– Subject's name

– Subject's unique identification number

Label
Name……
Age……
Specimen No……
Specimen Type……
Date……
Time……

Storage of Specimens

Store specimens at 4 °C before and during transportation within 48 hours

Store specimens at -70 °C beyond 48 hours.

Ziehl Neelsen Staining Procedure

The recommended method for staining mycobacteria is the Ziehl Neelsen but less sensitive. ZN is described as hot method of staining for mycobacteria, used to identify the acid fact organisms of mycobacteria. It is more sensible to make use of a weaker decolorizing agent for staining NTM as they may be more sensitive to the AFB decolonization procedure. Finally smear staining was done by standard ZN Method for AFB (Figure 1):

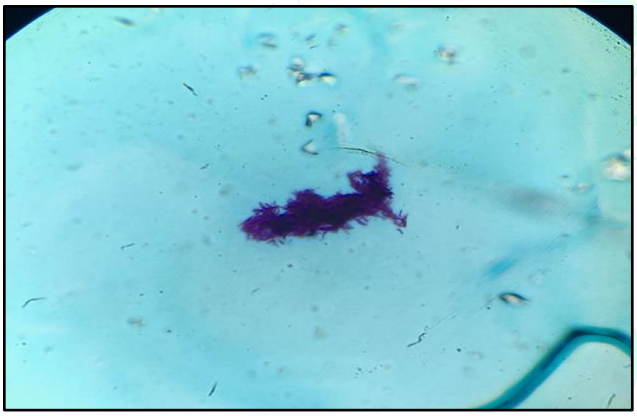

Figure 1 AFB in culture smear (ZN staining)

Mycobacterial Culture Processing

Process of samples were centrifuged and were directly inoculated using syringe into the BacT/Alert MP vials of the BacT/Alert 3D system (bioMerieux, France) which restraining modified Middlebrook 7H9 with supplement of an antibiotic containing (azlocillin [0.0034%, wt/vol] , vancomycin [0.0005%, wt/vol]), polymyxin B [10,000 U], nalidixic acid [0.04%, wt/vol], trimethoprim [0.00105%, wt/vol] and amphotericin B [0.018%, wt/vol] (Figure). And also inoculate processed samples (0.2 ml) in 3% LJ medium and cultured for eight weeks in an incubator at 35-37°C under 5-10% CO2 (Figure 7). BacT/Alert 3D vials was continuous observed by the BacT/Alert 3D system. BacT/alert system shows positive vials for the existence of acid fast bacilli (AFB) were subjected to smear microscopy (Figure 8). No growth after 42 days of incubation was treated as negative growth for mycobacteria. Then positive culture was further identified by phenotypic as well as genotypic.

Cultures with positive growth on the BacT/ALERT 3D and presence of AFB by ZN stain were screened with biochemical tests which included niacin production, catalase activity at 68 C at pH 7, and were tested with a rapid TB antigen assay (SD-Bioline Ag MPT64 Rapid TM assay; Standard Diagnostics, Kyonggi-do, Korea) which identifies antigens specific to MTBC. Isolates confirmed as MTB then went through a drug susceptibility test with the polymerase chain reaction based Genotype MTBDR plus test (Hain Lifescience, Nehren, Germany). Cultures with positive growth on BacT/ALERT 3D and the presence of AFB by ZN stain but that were negative for MTBC using the SD-Bioline assay were further identified for the species level.

Species Identification of NTM

Characterization of species was carried out with the reverse hybridization-based line probe assay as per the manufacturer's instructions.

RESULTS

In this study, 3320 sputum specimens, suspected cases of tuberculosis were included for the presence of mycobacterium species. Out of 3320 samples, Smear microscopy test showed 512 (15.4 %) positive for AFB by ZN staining. Further, 652 (19.6%) cases were BacT/ALERT® MP culture positive.

Culture positive isolates were subjected for species identification with the help of several phenotypic and genotypic methods, which results in 189 (29%) as NTM and 463 (71%) as MTBC isolates.

Clinical Symptoms of Pulmonary NTM Patients

Respiratory symptoms are most frequent among pulmonary patients including cough dyspnoea, hemoptysis, fever, weight loss, fatigue .Out of 189 sputum NTM positive cases have 122 (64.5%) cough, hemoptysis 27 (14%), dyspnoea 95 (50%), Fatigue 89 (47%) , 55 weight loss (50%) and fever 57 (30%) Table 1.

Table 1: Clinical symptoms of pulmonary infected ntm patients

Symptoms	N= 189%
Cough	122 (64.5%)
Dyspnoea	95 (50%)
Fatigue	89 (47%)
Fever	57 (30%)
Hemoptysis	27 (14 %)
Weight loss	55 (26%)

NTM Species in Sputum Isolates

M. abscessus 59, *M. fortuitum* 38, *M. intracellular* 25, *M. avium* 16, *M. chelonae* 14, *M.simiae* 9, *M.interjectum* 9, *M.gordonae* 6, *M.kanasii* 5 *M.szulgai* 4 , *M.malmoense* 2, *M.intermedium* 1, *M. scrofulaceum* 1 species were reported from respiratory samples. Whereas *M. abscessus and M. fortuitum* were frequently isolated from Sputum specimen from pulmonary infections

Additionally, we observed in our study that 42 (22%) patients were smokers, while 189 (78%) were non-smokers. A total of 57 (30%) out of 189 isolates were TB retreatment patients, infected with NTM. In our study, 125 (66%) NTM infected patients were married whereas 64 (33.8%) who were not presently married.

DISCUSSION AND CONCLUSION

NTM infections are an emerging global public health problem, especially in developing world. NTM and MTBC were proven to be responsible for various lungs, soft and

skin tissues, and disseminated infections. Microscopy of AFB is rapid; however it does not discriminate between MTBC and NTM. Biochemical tests are time taking and interpretation of results may be difficult. The GenoType® Mycobacterium CM/AS assay is reliable and rapid detection method in species of mycobacteria, which can give patients with the onset of early targeted therapy. We have reported in this study, the prevalence of NTM and its species diversity addresses in respiratory and non- respiratory samples gained from population of Northern India.

In our study, most recurrent NTM species reported in sputum specimens among pulmonary disease was *M. abscessus,* this suggests its high prevalence could be the result of its abundance in this region and its feature of drug resistance and pathogenicity leads to its persistence in this area.

In most part of India records of NTM was reported very less. The reason for this may be lack of awareness in the unorganized laboratory structure, limited funds, other burden of disease, in the hospital as well in rural settings among clinicians and microbiologist especially.

The higher occurrence of NTM and its species diversity suggests the urgent need and exact characterization including antibiotic susceptibility connected with NTM required for appropriate management and treatment of patients.

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