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Full Length Article

Prevalence and species spectrum of both pulmonary and extrapulmonary nontuberculous mycobacteria isolates at a tertiary care center

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ABSTRACT

Objective/background: Nontuberculous mycobacteria (NTM) infection associated with pulmonary and extrapulmonary disease has been increasing globally. Despite an increase in incidence rate of NTM infection, its prevalence, species diversity, and circulation pattern in India is largely unknown. This study sought to investigate the overall burden and diversity of NTM among both pulmonary and extrapulmonary clinical isolates from a Northern Indian population. *Methods:* The study was conducted in the Department of Microbiology, from January 2013 to December 2015. A total of 4620 clinical samples were collected from patients suspected to have pulmonary and extrapulmonary tuberculosis. Preliminary diagnosis was performed using Ziehl–Neelsen staining followed by liquid culture in BacT/ALERT three-dimensional system. A total of 906 positive cultures obtained were differentiated as either NTM or *Mycobacterium tuberculosis* complex using a biochemical and MPT64 antigen test. Further identification of NTM species was confirmed with a line probe assay. *Results:* Out of 906 cultures isolates, 263 (29.0%) were confirmed as NTM and 643 (71.0%) were identified as *Mycobacterium tuberculosis* complex. A total of 79.4% of the NTM were recovered from pulmonary and 18.2% from extrapulmonary specimens. The diversity of NTM species was high (13 species) and predominated by *Mycobacterium abscessus* (31.3%) followed by *Mycobacterium fortuitum* (22%), *Mycobacterium intracellulare* (13.6%), *Mycobacterium chelonae* (9.1%), however, *M. abscessus* and *M. fortuitum* were the predominant species in both types of clinical isolates. Men (60.4%) and older patients aged greater than 55 years were the predominated risk group for NTM infection. *Conclusion:* The high prevalence and species diversity of NTM suggests the need for immediate and accurate characterization of NTM for proper treatment and management of patients.

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Introduction

Nontuberculous mycobacteria (NTM) has been identified in human pulmonary and extrapulmonary diseases and are of great concern for clinicians and microbiologists because of their increasing global incidence [1]. An increasing pattern of NTM was recently reported from India with a prevalence rate from 0.7% to 34% [2,3]. Geographical variance has also been documented in both prevalence and species spectrum of NTM. *Mycobacterium avium*, *Mycobacterium fortuitum*, and *Mycobacterium scrofulaceum* are the most frequently occurring species as major etiological agents in human infections out of 42 disease-causing NTM species among all known NTM species [2,4]. In India, fewer studies have been documented for the species wise prevalence of NTM in pulmonary and extrapulmonary specimens. Depending on the species isolated and its susceptibility profile, the treatment options differ [5]. Additionally, most of the NTMs are resistant to the drugs and antibiotics that are being used to treat the patients infected with *Mycobacterium tuberculosis* complex (MTBC). Studies regarding this aspect are scarce in our geographical location. Therefore, the determination of the species spectrum of NTM at a particular geographical location is very necessary.

False diagnosis of NTM as tuberculosis (TB) or multidrug resistant-TB (MDR-TB) poses a significant challenge for developing an effective patient care strategy. Conventional methods like biochemical tests for NTM diagnosis are labor intensive, error prone, as well as requiring safety procedures to perform them [6]. However, newer techniques like high performance liquid chromatography, chemiluminescent DNA probe, nucleic acid amplification, and sequencing of 16S ribosomal RNA genes are quite sensitive but require highly sophisticated and expensive instruments which limits their utility in clinical diagnosis. However, the GenoType Mycobacterium common mycobacteria/additional species assay is rapid and can accurately differentiate different NTM species. With this background, the objective of our study is to estimate the prevalence of NTM among pulmonary and extrapulmonary isolates and differentiate the NTM species among both types of pulmonary and extrapulmonary isolates at this geographical part of the country.

Materials and methods

Study site, design, and ethical clearance

The prospective study was performed at the Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow. TB suspected patients from visiting indoor patient departments, outdoor patient departments, and various wards between January 2013 and December 2015 were screened for the study. The study was approved by the Institutional Ethical Committee of the Institute and a written informed consent was obtained from all patients prior to their recruitment in the study.

Data collection

Approximately 2–10 mL of respiratory (sputum, bronchoalveolar lavage fluid, and lung tissue) and nonrespiratory specimens (lymph node aspirate, cold abscesses, pleural fluid, cerebrospinal fluid, synovial fluid, ascetic fluid, urine, gastric aspirate, pus, bone marrow, wound, and pus swab) were collected consecutively from 4620 nonrepeated patients having extrapulmonary and pulmonary TB. Patients who belong to north Indian ethnicity were enrolled for the present study. Patients of all age groups were considered for the study and included both male and female populations.

Microbiological methods

All the clinical specimens received were subjected to smear microscopy using the Ziehl–Neelsen (ZN) staining method [7]. Specimens, which contain normal commensal bacterial flora, were decontaminated with the standard N-acetyl-L-cysteine-NaOH method [8]. Specimens were centrifuged and the sediment was inoculated into the vials of the BacT/ALERT three-dimensional (3D) system (BioMerieux, Marcy l'Etoile, France) containing modified Middlebrook 7H9 with an antibiotic supplement (amphotericin B: 0.018% weight/volume [wt/vol]; azlocillin: 0.0034% wt/vol; nalidixic acid: 0.04% wt/vol; trimethoprim: 0.00105% wt/vol; polymyxin B: 10,000 U; and vancomycin: 0.0005% wt/vol). BacT/ALERT 3D vials were monitored continuously with the BacT/ALERT 3D system [9]. Samples with positive growth vials were removed from the machine and then were subjected to smear microscopy for the presence of acid fast bacilli (AFB). Samples that failed to show any growth after 6 weeks of incubation in the machine were removed and treated as negative for mycobacteria.

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.

NTM species identification

Characterization of species was carried out with the reverse hybridization-based line probe assay as per the manufacturer's instructions [10]. Genotype CM (Hain Lifescience, Nehren Germany) was used for primary identification of the most common mycobacterium species. Unidentified isolates or additional mycobacterium species from the above assay was performed using Genotype Mycobacterium AS kit (Hain Lifescience, Nehren, Germany). Interpretation of the final results after hybridization was done based on the presence and absence of different bands and compared with reference band as provided in the kit.