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RESEARCH ARTICLE

NON-TUBERCULOUS MYCOBACTERIA IDENTIFICATION IN SPECIES LEVEL FROM RESPIRATORY SPECIMENS IN SOUTH INDIA

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ABSTRACT

Non-tuberculous mycobacteria (NTM) is a term used for a huge number of potentially pathogenic and non-pathogenic environmental mycobacterial species other than Mycobacterium *tuberculosis* complex (MTBC) and Mycobacterium leprae. Aims and Objectives: To identify the species on NTM isolated from the sputum samples of the base line surveillance study (BS study) by high performance liquid chromatographic (HPLC) method. Methods: This was a base line surveillance study carried out from model DOTS programme implemented in Thiruvallur. Base line survey has been completed at the Government Hospital Thiruvallur, Chennai, during the period April 2009 to July 2011. The clinical evaluation of 98 sputum samples were collected and processed for culture by modified pertroff method. After culture selection criteria for non-mycobacteria were identified to species level using HPLC analysis were done by standard operating procedure. Results: A total of 795 cultures of NTM were undergone HPLC identification 98 among them M.*terrrae* was predominant 42 out of 92 followed by M.*avium* (12) , M.*chitae* (10), M.*fortuitum* (7), M.*farcinogenes* (6), M.*simiae* (5), M.*fortuitum complex* (4), M.*bovis* (3), M.*flavescens* (3), and the unidentified 6 was noticed. Conclusion: It concludes the rate of NTM from respiratory specimens at our area has been increasing steadily and therefore, early differentiation between pulmonary TB and NTM lung disease in patients with AFB smear-positive specimens is necessary. Additionally our study says patient presenting with features of TB is less likely to have NTM disease, in related settings.

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INTRODUCTION

Pulmonary infections due to nontuberculous mycobacteria (NTM) are increasingly recognized worldwide. Of 95 species of NTM, around a third are associated with human infections (1). Improved diagnostic methods and increased physician awareness might have led to such increase in NTM infection. In addition, it has been suggested that its real prevalence is increased due to various causes such as changing demographics, with aging populations, increased co-morbidities, and immunosuppression (2). However, epidemiologic characteristics of NTM infection remain largely unclear. More than 125 species of NTM have been catalogued and available online out of which at least 42 species associate with disease in humans (3). The NTM were first recognized as human pathogens in the year 1950 and since then more than 170 NTM species have been identified and speciated (4) NTM was initially recognized as important only in 1982, when Mycobacterium avium complex (MAC) was isolated and considered as the most common opportunistic bacterial infection in AIDS patients (5). NTM are ubiquitous organisms found in the environment as saprophytes and are emerging as main cause of infectious diseases worldwide (6). NTM species also differ in their pathogenicity, with a higher propensity to cause disease in patients with impaired immunity. This can be either locally impaired immunity due to pre-existing lung disease or systemic, such as with haematological malignancy, immunosuppressive treatment or HIV/AIDS (7). NTM are divided into slow growing and rapid growing based on their growth rate in culture. *Mycobacterium terrae* was first isolated by Richmond (8) and Cummings in 1950 from radish washings, which was originally simply called as the "radish bacillus" (Richmond). Likewise *Mycobacterium intracellulare (M. intracellulare)* and *Mycobacterium avium (M.avium)* subspecies, encompassed within the *Mycobacterium avium* complex (MAC), are slow-growing mycobacteria requiring more than seven days to form mature colonies on subculture (9) Also the chances of missing NTM species are higher in TB-endemic countries, which are poorly equipped and overburdened with other diseases (10).

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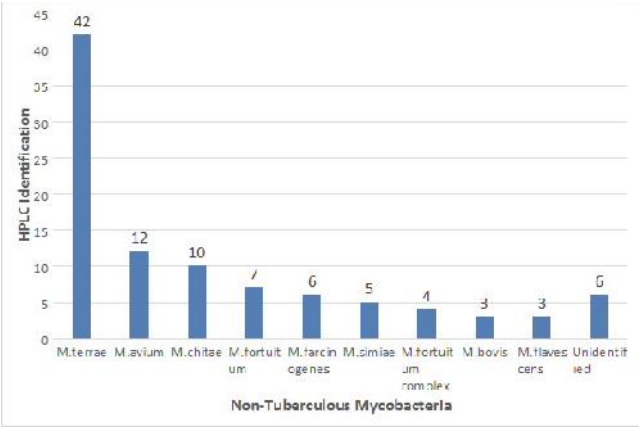
Several species of pathogenic mycobacteria, including M. avium and M. marinum, have been shown to invade and replicate within a variety of human nonprofessional phagocytes, such as fibroblasts and human epithelial cells (11).Hence, the present study was to identify the species on NTM isolated from the sputum samples of the base line surveillance study (BS study) by HPLC method, Southern India.

MATERIALS AND METHODS

This was a base line surveillance study carried out from model DOTS programme implemented in Thiruvallur. Base line survey has been completed at the Government Hospital Thiruvallur, Chennai, during the period April 2009 to July 2011 done at National Institute for Research in Tuberculosis (NIRT), Bacteriology Laboratory. After cultures isolates were identified as 98 non-tuberculous mycobacteria, cultures were collected and processed for isolation and identification of mycobacterium. After the selection criteria for Non-mycobacteria & mycolic acid extraction were identified to species level using HPLC analysis were done by standard operating procedure and performed at the National Institute for Research in Tuberculosis, Chennai, using a previously described protocol (12). After sub-culturing on solid LJ media. Each batch of the culture was accompany with positive and negative control tube. The confirmed NTM isolates were further speciated by pigment production, initial the biochemical tests, i.e. Niacin, para- nitro benzoic acid (PNB) test to differentiate the growth into MTBC and NTM. The NTMs were further identified to species level by morphological character and biochemical tests, i.e growth morphology, growth rate, growth at 25°C, 37°C and 44°C, pigment production in dark (schotochromogen), pigment production on exposure of light (photochromogen), no pigment production (non-chromogen),were noted (Myneedu 5). The prepared samples were subjected to HPLC analysis using UV detector. The HPLC peaks, which were shown between low- and high-molecular weight standards, were first separated into single, double, triple, and multiple cluster patterns. Each cluster group was identified by its number of peaks, retention times, and relative peak heights.

RESULTS

The non-tuberculous mycobacteria cultures were undergone HPLC identification and tested 795 patients' isolates. In the LJ media, 795 mycobacteria culture were identified as NTM including from sputum 98 samples. The microbiological characteristics of these patients' cultures 18% were pigmented, all Para-nitro benzoic acid (PNB) culture growth were positive. All mycobacterial strains were identified as *Mycobacterium tuberculosis* by other means than the niacin test (negative), and all catalyst are positive. But 2 of them Niacin positive; one is m.simiae & another one mixed growth were identified. The most commonly isolated mycobacterial species were the total of 98 cultures of NTM were undergone HPLC identification among them M.*terrrae* was predominant 42 out of 92 followed by M.*avium* (12) , M.*chitae* (10), M.*fortuitum* (7), M.*farcinogenes* (6), M.*simiae* (5), M.*fortuitum complex* (4), M.*bovis* (3), M.*flavescens* (3), and the unidentified 6 was noticed. High-performance liquid chromatography patterns, which did not match with standard Mycobacterium species, were included as 'unclassified' NTM.



DISCUSSION

NTM have been increasingly documented as an important cause of morbidity in the developing countries. The identification of NTM is important because positive microscopy cannot differentiate *M. tuberculosis* complex from NTM infection, causing diagnostic and clinical dilemmas (25). Non-tuberculous mycobacteria infections occur usually in patients with pre-existing lung disease, (13&14) healed cavities from previous TB and in those with reduced immunity. Recent study revealed that NTM was more probable to infect older females as accounted previously (15). This may hint that estrogen has a self-protective role against NTM (16). However, in other studies, no major variation of distribution was found among genders (17 & 18) and the occurrence of NTM in younger individuals should also be given concentration (19). The difference of prevalence among genders possibly due to the heterogenicity of the contagion-causing microorganism among different regions (17) or the distribution of ages among genders (16). In the past decade, there has been an increase in the reporting of NTM disease (20) but most of these reports are from the industrialized world and approximately 200 NTM species have been described and all of these may not be clinically relevant. There is a scarcity of NTM reporting from the developing countries with high TB burden. Only a handful of studies have been published from India (4).

The prevalence and incidence of lung disease by *Mycobacterium terrae* has been increasing worldwide since the first reported case of pulmonary infection in 1983 (21). Whereas the present study observes the *M. terrae* (n=42) was most predominantly observed these may be environmental mycobacteria growing in humidity damaged structure (22). The *M. terrae* infection can cause progressive debilitating disease. Members of *M.terrae* complex (*M. terrae*, *M. nonchromogenicum* and *M. triviale*) may be associated with mycobacterial disease (1) & Jesudason *et* al (23) from South India observed that *M. chelonae* and *M. fortuitum* accounted for 67% of the total NTM isolates along with others, i.e *M. szulgai, M. terrae, M.scrofulaceum, M. flavescens, M. gordonae, M.simiae* and *M. smegmatis.* Also noted the Jesudason due to NTM have been widely reported in immune-compromised individuals as AIDS patients (1). This is similar observation with results from a study in North India, which found that *M. fortuitum* and *M. intracellulare* were the most common isolates (24). In the other study from in north India indicates, *M. intracellulare* was the most common NTM isolate followed by *M. abscessus* among pulmonary patients.

The most frequently NTM species isolated from Delhi and Kasauli was *M. avium* intracellulare (8.6%) from sputum specimens (5 & 19). In controversy of most Indian studies *M. tuberculosis* has been found as major cause of mycobacterial infections and the proportion of NTM has been considered low. Species like *M.fortuitum, M. avium, M. scrofulaceum etc*., have been isolated in different studies (25, 26,27&28). NTM isolation rates are reported to range from 0.5 to 8.6% in India (29). A recent study from central India reported prevalence of NTM increased from 1.0% in 2005 to 3.5% in 2008 and 88.6% of the NTM isolated were clinically relevant (29). The present study shows the prevalence of *M. fortuitum* 7 has identified because of the reasons for treatment failures are many, but one reason could be an erroneous diagnosis of TB due to dependence on only smear microscopy for initial diagnosis of the disease. This could be due, in part, to the presence of NTM in sputum samples, and the fact that smear microscopy is not an effective tool to differentiate between NTM and MTB. Whereas the previous study shows that M**.** *abscessus* and *M. fortuitum* are usually associated with cutaneous infections but are also unusual causative agents for pulmonary disease and lymphadenitis (30). The source of *M. simiae*, *M. malmoense*, and *M. haemophilum* is still uncertain (31). The most common species of NTM in our samples was *M. abscessus*. This is in contrast with results from a study in North India, which found that *M. fortuitum* and *M. intracellulare* were the most common isolates (24). Infection with NTM, when it occurs, is thought to be due to exposure to these environmental sources (32).

High lights of our study

All samples are collected for this study pulmonary only, and study done by BCG Trial area and the samples are processed for HPLC Methods. Majority of recent studies for NTM species process for extra pulmonary samples but present study only from pulmonary samples.

Conclusion

It concludes the rate of NTM from respiratory specimens at our area has been increasing steadily and therefore, overall rapid identification and differentiation to species level by molecular assay may help in targeted therapy and management of infections and NTM lung disease in patients with AFB smear-positive specimens is necessary. Also, our study says patient presenting with features of TB is less likely to have NTM disease, in related settings.

Conflict of interests

All authors declare no conflict of interest

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