



## NON TUBERCULOUS MYCOBACTERIA ISOLATED FROM CLINICAL SPECIMENS AT A TERTIARY CARE HOSPITAL IN SOUTH INDIA

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### Abstract

**Purpose:** This is a retrospective analysis of the isolation rates of nontuberculous mycobacteria (NTM) from various clinical specimens and their antimicrobial susceptibility patterns. **Methods:** All NTM isolated between 1999 and 2004 at Christian Medical College, Vellore, South India, were identified with various biochemical tests. Antimicrobial susceptibility test for all NTM was performed by standard methods. **Results:** A total of 32,084 specimens were received for culture, of which 4473 (13.9%) grew acid fast bacilli (AFB). Four thousand three hundred (96.1%) of the AFB were *M. tuberculosis* while 173 (3.9%) were NTM. Of the 173 NTM, 115 (66.5%) were identified to the species level. Pus, biopsy specimens and sputum specimens yielded most of the NTM of which *M. chelonae* (46%) and *M. fortuitum* (41%) accounted for majority of them. *M. chelonae* and *M. fortuitum*, showed highest susceptibility to amikacin (99.2%). NTM were repeatedly isolated from seven sputum specimens, 15 biopsy and pus specimens, two CSF and two blood cultures. Six were isolated from patients with AIDS and five from post transplant patients. **Conclusions:** The isolation of NTM from various clinical specimens is reported in this study to highlight the associated diseases and therapeutic options in these infections.

**Key words:** NTM, *M. chelonae*, *M. fortuitum*

Although *M. tuberculosis*, *M. bovis* and *M. leprae* are established pathogens predominating human mycobacterial infections, nontuberculous mycobacteria (NTM) are increasingly being reported as etiological agents of human infections.<sup>1</sup> NTM comprising of over 95 species are naturally seen as saprophytes<sup>2</sup> but are known to cause four different categories of infections in humans such as, i) pulmonary infections resembling tuberculosis, ii) extra pulmonary infections affecting lymph nodes, skin and soft tissue, iii) multifocal disseminated infections and iv) infections in immunocompromised individuals such as AIDS and transplant patients.<sup>3</sup> The growing population of HIV infected individuals and other immunosuppressed / immunocompromised patients coupled with better diagnostic techniques has led to an increase in the number of NTM being reported in human infections in recent years.<sup>4</sup>

NTM produce infections more commonly in the presence of predisposing factors / underlying diseases; they are also notably resistant to commonly used antitubercular drugs. These factors augment morbidity and limit therapeutic options in such infections. NTM have been reported world wide with varying frequencies,<sup>5-8</sup> while in India isolation rates are between 0.7% and 34%.<sup>9</sup> We have reviewed the isolation rates of NTM from various clinical specimens over a period of six

years at the department of Clinical Microbiology, Christian Medical College, Vellore – South India. Through this study we wish to emphasize the need to look for NTM in various clinical specimens.

### Materials and Methods

This study includes the isolation of NTM from all clinical specimens from suspected pulmonary and extra pulmonary tuberculosis received between 1999 and 2004. Early morning well coughed out sputum specimens, broncho alveolar lavage, bronchial wash and endotracheal aspirate specimens were received from patients with clinical and radiological findings suggestive of pulmonary tuberculosis. From children and patients from whom expectorated sputum was not possible, gastric juice was collected. Biopsy and pus specimens were obtained from patients with lymphadenitis, abscesses or sinuses of chronic or recurrent nature. Entire early morning urine specimens were received from patients suspected of urinary tuberculosis. Sterile body fluids such as cerebrospinal fluid (CSF), blood, pleural fluid and others were collected from patients suspected of disseminated mycobacterial infections. All specimens were collected with aseptic precautions in sterile leak proof containers and transported to the laboratory.

The specimens were processed on the same day for microscopy and culture by standard procedures.<sup>10</sup> The specimens from 'unsterile' sites such as sputum, urine and gastric juice were decontaminated by the Petroff's method.<sup>10</sup> Sterile body fluids such as CSF, blood, pleural fluid and other fluids were processed without decontamination but after

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sterility check. Specimens in large volumes were centrifuged at 3000 rpm for 30 minutes and the deposit was used as inoculum. Biopsy specimens were ground with sterile precautions and processed. Smears were made and stained with auramine O and specimens were inoculated on to Lowenstein Jensen (LJ) media in duplicate and incubated at 37 °C. The cultures were examined everyday for one week and thereafter once a week for 6 weeks.

Smears were made from colonies on LJ medium and stained by Ziehl Neelsen stain. When colony morphology and smear morphology were suggestive of *M. tuberculosis*, they were further confirmed by Niacin test.<sup>10</sup> Those suspected to be NTM were subjected to a battery of biochemical tests for identification; these included nitrate reduction, aryl sulphatase (3 and 14 days), ability to grow in the presence of 5% NaCl, growth on MacConkey agar, susceptibility to polymyxin B 300 units, pigment production, rate of growth, growth at 30 °C, 37 °C, and 45 °C, tween 80 hydrolysis, potassium tellurite reduction, urease production, iron uptake and semi quantitative catalase.<sup>11</sup> Antimicrobial susceptibility testing for the rapidly growing NTM was carried out on Mueller Hinton agar by the disc diffusion Kirby Bauer method<sup>12</sup> to the following antibiotics: chloramphenicol (10 µg), erythromycin (15 µg), tetracycline (30 µg), vancomycin (30 µg), ciprofloxacin (5 µg), amikacin (30 µg), ofloxacin (5 µg), piperacillin (100 µg), cotrimoxazole (25 µg), rifampicin (5 µg), streptomycin (10 µg), gentamicin (10 µg), polymyxin B 300, SSS (300 µg). For the slow growing NTM susceptibility to streptomycin, ethambutol, isoniazid and rifampicin were tested by the resistance ratio method in LJ medium.<sup>13</sup> The organisms were inoculated onto antibiotic containing media and the standard reference H<sub>37</sub>RV strain was included in each batch. The ratio of the MIC of the test strain to the MIC of the standard H<sub>37</sub>RV strain gave the resistance ratio based on which susceptibility profile was deduced.<sup>13</sup>

## Results

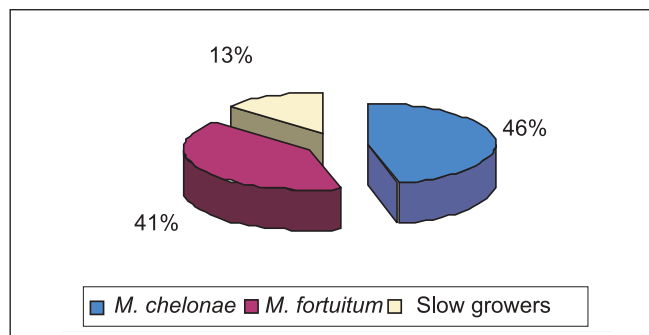
Of the 32,084 specimens received for mycobacteria culture during the study period of six years 4473 (13.9%) grew acid fast bacilli (AFB). Four thousand three hundred (96.1%) of the acid fast bacilli were *M. tuberculosis* while 173 (3.9%) grew NTM from various clinical specimens with a presumptive diagnosis of mycobacterial infection. Of the 173

NTM 115 (66.5%) could be identified to the species level with the tests mentioned earlier, while the remaining could not be speciated.

Isolation of NTM from pulmonary and other specimens is shown in the table. Surgical specimens such as pus and biopsy specimens yielded largest number of NTMs followed by the respiratory specimens. *M. chelonae* (46%) and *M. fortuitum* (41%) accounted for the majority of the isolates (Figure 1).

The median age of patients from whom NTM were isolated was 28 years in patients between 16 and 40 years, while in patients between 41 and 70 years it was 60 years. Sixty four (56%) were isolated from males and 51 (44%) were isolated from females. There was no seasonal variation in the isolation rates of NTM in all the six years.

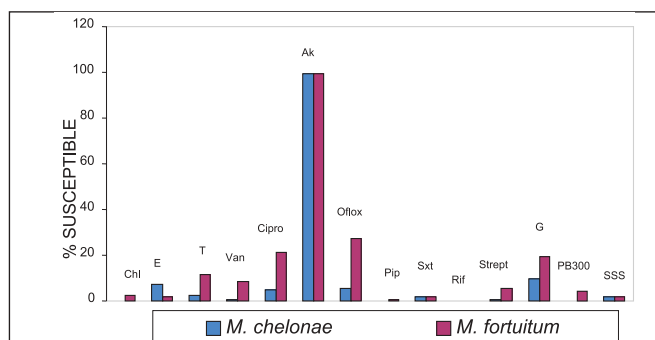
Antibiotic susceptibility testing done for the rapid growers namely *M. chelonae* and *M. fortuitum*, showed highest susceptibility to amikacin (99.2%). Only one isolate of *M. fortuitum* and *M. chelonae* each were resistant to amikacin. Susceptibility to ofloxacin, ciprofloxacin and gentamicin in the case of *M. fortuitum* were 27%, 21% and 19.2% respectively but *M. chelonae* showed much lower susceptibility rates to these. Both the organisms exhibited very low susceptibility rates to the other antibiotics tested (Figure 2). Of the slow growers one strain of *M. terrae* was susceptible to all four drugs tested while 3 strains of *M. szulgai* and one strain of *M. gordonae* were susceptible to only ethambutol. All the others were resistant to all four drugs tested (streptomycin, ethambutol, isoniazid and rifampicin).



**Figure 1:** Isolation NTM from clinical specimens

**Table: Distribution of NTM from different clinical specimens**

Specimens	<i>M. chelonae</i>	<i>M. fortuitum</i>	<i>M. szulgai</i>	<i>M. terrae</i>	<i>M. scrofulaceum</i>	<i>M. flavescens</i>	<i>M. gordonae</i>	<i>M. simiae</i>	<i>M. smegmatis</i>
Biopsy	24	19	-	1	1	1	-	-	-
Sputum	8	6	2	1	-	-	1	1	1
Pus	15	18	-	-	-	-	-	-	-
CSF	3	-	-	-	-	-	-	-	-
Gastric juice	-	3	1	-	-	-	-	-	-
Urine	-	-	-	-	-	-	-	-	1
Blood	3	1	-	-	-	-	-	-	-
<b>Total</b>	<b>53</b>	<b>47</b>	<b>3</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>1</b>	<b>2</b>



**Figure 2:** Antibiotic susceptibility patterns of NTM

## Discussion

NTM, also known as atypical mycobacteria, MOTT bacilli, environmental mycobacteria and opportunistic mycobacteria are saprophytes naturally distributed in soil, water and dust.<sup>2</sup> Nevertheless, these organisms have been reported to cause a variety of infections, more so in immunocompromised / immunosuppressed individuals and to a lesser extent in immunocompetent individuals. The incidence of tuberculosis has reduced in developed countries but infections due to NTM is on the rise,<sup>17</sup> while in developing countries like India, tuberculosis is still a major health problem; NTM are also reported frequently as causative agents of human infections.

Respiratory infections due to NTM are often associated with various conditions such as chronic obstructive pulmonary disease, cystic fibrosis of lung, bronchiectasis, emphysema of lung, previously treated pulmonary tuberculosis and lung cancer. Skin and soft tissue infections are frequently associated with post surgical wounds and injection abscesses. Other predisposing conditions include diabetes mellitus, leukemia, systemic lupus erythematosus, chronic alcoholism, AIDS and transplant patients.<sup>2, 4</sup>

*M. kansasii*, *M. scrofulaceum*, *M. fortuitum*, Mycobacterium avium complex, *M. xenopi* and *M. simiae* have been reported to cause pulmonary infections.<sup>2</sup>

In our study, *M. chelonae* and *M. fortuitum* accounted for 67% of NTM isolated from respiratory specimens. *M. fortuitum*, *M. chelonae* and *M. marinum* have been commonly reported to cause skin and soft tissue infections, joint and bursae infections, wound infections and injection abscesses.<sup>2</sup> In this study *M. chelonae* and *M. fortuitum* were the predominant isolates from pus and biopsy specimens of patients with soft tissue infections and injection abscesses. Disseminated infections due to NTM have been widely reported in immunocompromised individuals especially in AIDS patients.<sup>2</sup>

In our study, only 7 (4%) isolates were from blood and CSF that could be considered as disseminated infections.

Since NTM are ubiquitous in nature and a possible

laboratory contaminant, the isolation of these organisms from specimens should meet certain criteria to confirm their etiological significance such as, a) repeated isolation of the same organism from a patient, b) associated positive clinical and radiological evidence and c) histopathological confirmation. These parameters need to be considered while reporting NTM from clinical specimens. However, certain other parameters like a) collection of appropriate specimens directly from the lesion such as biopsies and BAL, b) isolation from sterile body fluids such as blood, CSF, pleural fluids, c) presence of any predisposing factors / underlying diseases and d) the immune status of the patient aids in assessing the etio pathogenesis of NTM when isolated.<sup>2,4</sup> In our study NTM were repeatedly isolated from the sputum specimens of seven patients, biopsy and pus specimens from 15 patients, CSF specimens of two patients and blood cultures of two patients. Six were isolated from patients with AIDS and five were isolated from post transplant patients. Eight were isolated from sterile body fluids and 80 were from post surgical skin and soft tissue infections.

The management of NTM infections includes medical treatment with various antimicrobial agents based on susceptibility patterns and surgical treatment as in the case of lymphadenitis, skin or soft tissue infections. Since most of these organisms are resistant to commonly used antimicrobial agents, susceptibility testing becomes mandatory before instituting an effective therapy.

Karak *et al* from Kolkata, have reported a NTM prevalence of 17.4% from sputum specimens from patients with fibrocavitary pulmonary diseases,<sup>3</sup> this was comparatively higher than the reports of the other workers. Chakrabarthi *et al* from Chandigarh documented NTM isolation rate of 7.4% from various clinical specimens and *M. fortuitum* was the commonest isolate.<sup>9</sup> Paramasivam *et al* from Chennai, South India has reported 8.6% of NTM from sputum specimens of patients in BCG trial area. *M. avium* / *intracellulare* was the species most frequently isolated in their study.<sup>14</sup> Das *et al* reported isolation of 8.3% NTM from various clinical specimens from Delhi and Kasauli.<sup>18</sup> There has also been a report from our institution on the isolation of *M. fortuitum* from surgical specimens of patients with post operative wound infections, injection abscesses, skin and soft tissue infections.<sup>15</sup>

Although there are many reports from India, the exact disease burden of NTM infections still remains unclear in India. These infections are under diagnosed in many laboratories due to lack of facilities and expertise. These organisms can be often misidentified as *Corynebacterium* spp. or *Nocardia* spp.<sup>16</sup> Newer molecular methods such as gene probes, PCR and DNA fingerprinting may be better diagnostic tools.

In conclusion, regular documentation and reporting of these NTMs from clinical settings along with their sensitivity

profiles is essential to be aware of the possible spectrum of diseases associated and preferred treatment options.

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