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Profile of Non-Tuberculous Mycobacteria in Progressive Pulmonary Disease in a Rural Tertiary Care Hospital in Central India Incorporating use of Gelatine Coated Face Masks for Collection of Samples

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Abstract: ***Background: Non - tuberculous mycobacteria (NTM) are environmental pathogens associated with biofilm formation and antibiotic resistance. The hydrophobicity of NTM results in preferential aerosolization from water, and many of these organisms are resistant to high temperature and are relatively resistant to low PH. After commitment of the Indian government to eliminate TB by 2025, extra - pulmonary TB has also gained equal attention along with paediatric TB and drug resistant (DR) TB. Aim and objectives: This study aimed to profile NTM species causing pulmonary disease in a rural tertiary care hospital in central India and evaluate the utility of gelatine - coated face masks for sample collection. Material and methods: A cross - sectional study was conducted from November 2019 to November 2021, involving 145 patients with progressive pulmonary disease. Sputum and gelatine mask samples were collected and analyzed. We have done Systematic random sampling in our study. Participants were selected from among patients visiting Medicine OPD or patients admitted under Medicine Department in rural Tertiary Care Hospital in Central India. Results: In the present study, Out of 121 cases of NTM culture group, majority 39 (32.2%) were in age group of 15 - 40 years. Mean age was 51.10 ±18.3 years ranging from 15 - 85 years. In gelatine mask group, out of 24 cases, majority 14 (58.3%) were in age group of ≥61 years. Mean age was 57.70±19.6 years ranging from 15 - 85 years. Majority 100 (82.6%) and 21 (87.5%) of cases in both the groups had breathlessness followed by cough in 83 (68.6%) and 16 (66.7%) cases respectively in culture and gelatine mask group. Majority cases had no history of tuberculosis in both groups respectively as 97 (80.2%) and 20 (83.3%) and 100 (82.6%) cases in culture group and 20 (83.3%) cases in gelatine mask group had no previous history of treatment of tuberculosis. No NTM growth was observed in either sample type. The study highlights the challenges in diagnosing NTM and underscores the need for better diagnostic tools. Conclusion: No NTM growth was observed in either sample type. Better diagnostic tools are needed as the currently available equipment for the diagnosis, species identification, and drug sensitivity testing of NTM are not fully developed.***

Keywords: Non - tuberculous mycobacteria, pulmonary disease, gelatine - coated face masks, rural healthcare, India

1. Introduction

Tuberculosis (TB) remains as one of the most serious challenge to public health. Under program conditions, for long time more attention was paid to pulmonary TB as sputum positive patients could spread disease to close contacts. Following the Indian government's commitment to eliminate TB by 2025, extra - pulmonary TB has gained equal attention alongside paediatric TB and drug - resistant (DR) TB.

Non tuberculous mycobacteria (NTM) are ubiquitous in the environment with the heaviest concentrations found in soil and water sources. They are associated with biofilm formation, which contributes to disinfectant and antibiotic resistance. The hydrophobicity of NTM results in preferential aerosolization from water, and many of these organisms are resistant to high temperature and are relatively resistant to low PH.[1]

As the rate of TB will reduce, the diseases caused by NTM will rise as has happened in the developed countries.[2, 3] A recent analysis showed that approximately half of those with positive NTM respiratory cultures fulfilled clinical criteria for active infection. In Kendall and Winthrop‘s review, the prevalence of NTM pulmonary infections based on laboratory records coupled with clinical characteristics varied between 4.1 and 14.1 per 100, 000 patient years. In patients greater than 65 years the prevalence was 47 per 100, 000 patient years. Women are also more likely to have NTM disease than men, the disease prevalence increases with age, and it is more common in the West and Southeast. In the United 6 States, Caucasians account for 90% of cases followed by Asians/Pacific Islanders and Blacks.[1]

For a long time, NTM were just considered as colonizers or commensals but after advent of Human immunodeficiency virus/Acquired immune deficiency syndrome (HIV/AIDS) they gained importance an important opportunistic organism. In AIDS patients with CD4 counts less than 200/cumm the chances of bacteraemia and disseminated disease were high and a number of studies from our centre has shown isolation from blood, sputum and stool samples of AIDS patients using a special technique named ―paraffin baiting.[4 - 9]

Four types of diseases are caused by NTM in humans. In broad terms, these four distinct clinical syndromes are: 1) Progressive pulmonary disease caused primarily by Mycobacterium avium complex (MAC), Mycobacterium abscessus subspecies, and Mycobacterium kansasii in USA; 2) Superficial lymphadenitis, especially cervical lymphadenitis, in children caused mostly in USA by MAC, Mycobacterium scrofulaceum, and in northern Europe Mycobacterium malmoense and Mycobacterium haemophilum 3) Disseminated disease in severely immuno - compromised patients and 4) Skin and soft tissue infection usually as a consequence of direct inoculation.[10, 11]

The present study was carried out with a scope to study the profile of non tuberculous mycobacteia in progressive pulmonary disease and use of gelatin coated face masks for collection of samples as it‘s a novel topic and very less literature is available for the same.

2. Materials and Method

This was a cross sectional study which was carried out in the Department of Medicine of a rural based teaching hospital from November - 2019 to November - 2021. Patients who are known cases of progressive pulmonary disease will be included in the study, irrespective of their sex. The patients from indoor patient department will be included in the study. Approval from the institutional ethics committee was sort before beginning the study. Participants were selected from among patients visiting the Medicine OPD or those admitted under the Medicine Department in a rural tertiary care hospital in central India. Inclusion Criteria: 1) Patients >12 years of age of either gender.2) Patients of progressive pulmonary disease (chronic obstructive pulmonary disease (COPD), bronchiectasis, pneumoconiosis, cystic fibrosis, and previous tuberculosis).3) Patients of drug - sensitive and drug resistant (pulmonary and extra - pulmonary) TB not responding to treatment. Exclusion Criteria: 1. Patients declining written informed consent.2. Patients unwilling to come back for follow up.

Based on the study conducted by Paramasivan CN et al. Prevalence of NTM disease among patients suspected of tuberculosis was 8.6% in Thiruvallur, 7.6% in Tambaram, 4.5% in Madras city and Bangalore each.100 Hence from open epi calculated sample size = with two - sided significance level (1 - alpha) - 95 absolute precision (l) = 5% in the formula, expected prevalence (p) = 8.6%, q=1 - p, Sample size n=3.84xpq/l2 =121. Considering non response/data loss = 20% =24 sample size was added to 121. Total sample collected was 145 study subjects.

We have done Systematic random sampling in our study. After taking informed consent and appropriate history patients was taken by same research associate. Symptoms pertaining to pulmonary disease were noted. Various signs were elicited and noted in the proforma. A predesigned pretested proforma was used to note the demographic and clinical features. Out of 145 cases, 121 samples were collected as sputum samples in Microbiology department and 24 samples were collected from gelatine coated face masks and processed same as sputum samples in Microbiology department. Gelatine face masks were kept for 12hrs in patients. X ray chest or high - resolution computed tomography scan was performed in all study subjects and findings consistent with NTM pulmonary infection on chest radiograph or high - resolution computed tomography scan like infiltrates (nodular or reticulonodular), cavities, multifocal bronchiectasis, and/or multiple small nodules were noted.

After collecting the data, entry was done in MS EXCEL Version 2020. IBM Statistical Package for Social Sciences version 26 (SPSS V.26.0) software was used for analysis. Socio demographic data was organized and presented by applying principles of descriptive statistics.

3. Results

Table 1: Distribution of study subjects according to age

Age groups (in years)	NTM Culture group n (%)	NTM Gelatine mask group n (%)
15 - 40	39 (32.2)	04 (16.7)
41 - 60	36 (29.8)	06 (25.0)
≥61	45 (38.0)	14 (58.3)
Total	121 (100)	24 (100)
Mean ± SD	51.10 ±18.3	57.70±19.6
Range	15 - 85	15 - 85

Table 1 shows distribution of study subjects according to age. Out of 121 cases of NTM culture group, The majority of cases, 100 (82.6%) and 21 (87.5%) in both groups, had breathlessness, followed by cough in 83 (68.6%) and 16 (66.7%) cases in the culture and gelatine mask groups, respectively. Out of 24 cases, majority 14 (58.3%) were in age group of ≥61 years followed 6 (25%) in age group of 41 - 60 years. Mean age was 57.70±19.6 years ranging from 15 - 85 years.

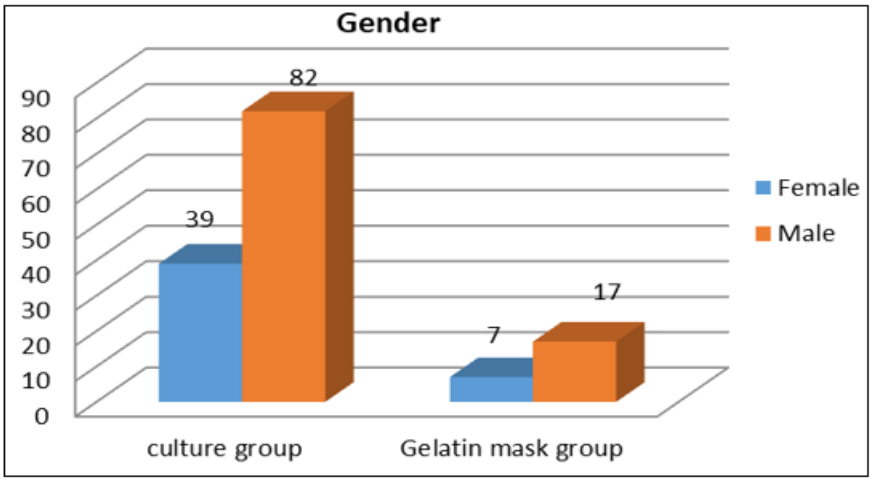

Figure 1: Distribution of study subjects according to sex

Figure 1 shows distribution of study subjects according to sex. Out of 121 cases in culture group, maximum 82 (67.8%) were males and rest 39 (32.2%). In gelatin mask group, 17 (70.8%) were males and rest 07 (29.2%) were females

Table 2: Distribution of study subjects according to presenting complaints

Presenting complaints	NTM Culture group n (%)	NTM Gelatine mask group n (%)
Fever	77 (63.6)	13 (54.2)
Breathlessness	100 (82.6)	21 (87.5)
Cough	83 (68.6)	16 (66.7)
Chest pain	26 (21.5)	06 (25.0)
Hemoptysis	07 (5.8)	00 (00)
Abdominal Pain	04 (3.3)	00 (00)

Table 2 shows distribution of study subjects according to presenting complaints. Majority 100 (82.6%) and 21 (87.5%) of cases in both the groups had breathlessness followed by cough in 83 (68.6%) cases and 16 (66.7%) cases respectively in culture and gelatine mask group. This was followed by fever in 77 (63.6%) culture group and 13 (54.2%) in gelatine mask group.

Table 3: Distribution of study subjects according to previous history of tuberculosis

Previous h/o TB	NTM Culture group n (%)	NTM Gelatine mask group n (%)
Yes	24 (19.8)	04 (16.7)
No	97 (80.2)	20 (83.3)
TOTAL	121 (100)	24 (100)

Table 3 shows distribution of study subjects according to previous history of tuberculosis. Majority cases had no history of tuberculosis in both groups respectively as 97 (80.2%) and 20 (83.3%).

Table 4: Distribution of study subjects according to previous history of treatment of tuberculosis

Previous H/o treatment	NTM Culture group n (%)	NTM Gelatine mask group n (%)
Yes	21 (17.4)	04 (16.7)
No	100 (82.6)	20 (83.3)
TOTAL	121 (100)	24 (100)

Table 4 shows distribution of study subjects according to previous history of treatment of tuberculosis. Majority 100 (82.6%) cases in culture group and 20 (83.3%) cases in gelatine mask group had no previous history of treatment of tuberculosis.

Table 5: Distribution of study subjects according to HIV status

HIV status	NTM Culture group n (%)	NTM Gelatine mask group n (%)
Positive	04 (3.3)	00 (00)
Negative	117 (96.7)	24 (100)
TOTAL	121 (100)	24 (100)

Table 5 shows distribution of study subjects according to HIV status. Out of 121 cases of culture group, 117 (96.7%) were negative for HIV and in gelatine mask group, all 24 cases were negative for HIV.

Table 6: Distribution of study subjects according to risk factors for Non tuberculous mycobacteria related to water

Risk factors	NTM Culture group n (%)	NTM Gelatine mask group n (%)
Drinks Bottle Water	04 (3.3)	00 (00)
Drinks City water	67 (55.4)	15 (62.5)
Drinks well water	52 (43.0)	09 (37.5)
Swims in Lake	10 (8.3)	01 (4.2)
Swims in Pool	03 (2.5)	01 (4.2)
Takes Daily Bath	86 (71.1)	18 (75.0)
Takes Shower	06 (5.0)	02 (8.3)

Table 6 shows distribution of study subjects according to risk factors for Non tuberculous mycobacteria related to water. Majority 86 (71.1%) cases in culture group and 18 (75%) in gelatine mask group had history of taking bath daily followed by 67 (55.4%) cases in culture group and 15 (62.5%) cases in gelatine mask group had history of drinking city water and 52 (43%) cases in culture group and 09 (37.5%) cases in gelatine mask group had history of drinking well water.

Table 7: Hematological parameters of study subjects

Mean Cytopathological parameters	NTM Culture group (Mean±SD)	NTM Gelatine mask group (Mean±SD)
Hemoglobin	10.84±2.8	11.91±2.6
Total leucocyte count	11.64±5.8	12.13±5.8
Mean lymphocyte count	13.31±9.3	14.44±8.6
Mean granulocyte count	80.49±12.8	78.37±10.1

Table 7 Shows mean hematological parameters in study subjects. Mean hemoglobin, total leucocyte count, mean lymphocyte count and mean granulocyte count in culture group was 10.84±2.8, 11.64±5.8, 13.31±9.3 and 80.49±12.8 respectively. Mean hemoglobin, total leucocyte count, mean lymphocyte count and mean granulocyte count in gelatine mask group was 11.91±2.6, 12.13±5.8, 14.44±8.6 and 78.37±10.1 respectively.

Table 8: Liver function test (LFT) parameters of study subjects

Mean LFT parameters	NTM Culture group Mean ± SD	NTM Gelatine mask group Mean ± SD
Total Bilirubin (mg/dl)	1.8±6.4	0.68±0.5
Conjugate bilirubin	0.85±5.3	0.17±0.1
Alanine transaminase (ALT)	56.17±98.4	27.33±22.9
Aspartate aminotransferase (AST)	62.85±100.4	39.70±29.9
Alkaline phosphatase (ALP)	89.78±43.3	95.41±54.9

Table 8 shows mean LFT parameters in study subjects. Mean total bilirubin, conjugate bilirubin, ALT, AST and ALP in culture group was 1.8±6.4, 0.85±5.3, 56.17±98.4, 62.85±100.4 and 89.78±43.3 respectively. Mean total bilirubin, conjugate bilirubin, ALT, AST and ALP in gelatine mask group was 0.68±0.5, 0.17±0.1, 27.33±22.9, 39.70±29.9 and 95.41±54.9 respectively.

Table 9: Kidney function test (KFT) parameters of study subjects

Mean KFT parameters	NTM Culture group Mean±SD	NTM Gelatine mask group Mean±SD
Sodium (meq/l)	132.47±13.9	130.54±8.6
Potassium	4.35±0.6	4.59±0.6
Urea	38.80±35.4	54.37±52.2
Creatinine	2.05±8.9	1.69±2.2

Table 9 shows mean KFT parameters in study subjects. Mean sodium, potassium, urea and creatinine in culture group was132.47±13.9, 4.35±0.6, 38.80±35.4 and 2.05±8.9 respectively. Mean sodium, potassium, urea and creatinine in gelatine mask group was 130.54±8.6, 4.59±0.6, 54.37±52.2 and 1.69±2.2 respectively.

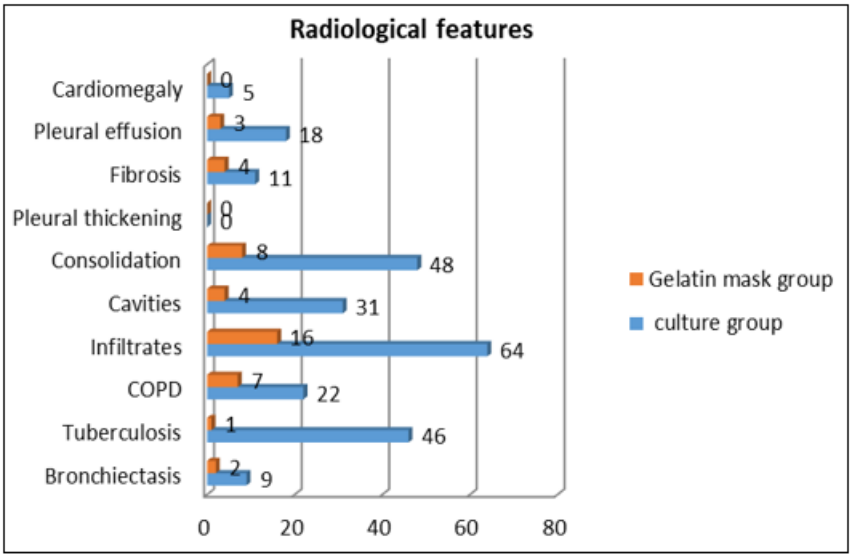

Figure 2: Radiological features in study subjects

Figure 2 shows radiological features in study subjects. In culture group, majority 64 (52.9%) had infiltrates followed by 48 (39.7%) cases had consolidation followed by 46 (38%) cases had tuberculosis on radiology. In gelatine mask group, majority 16 (66.67%) had infiltrates followed by 08 (33.3%) cases with consolidation followed by 07 (29.2%) cases with COPD on radiology.

Table 10: Culture growth in study subjects

Growth on culture	NTM Culture group n (%)	NTM Gelatine mask group n (%)
Yes	00 (00)	00 (00)
No	121 (100)	24 (100)
Total	121 (100)	24 (100)

Table 10 shows culture growth in study subjects. In both the groups we have done culture on LJ media for sputum samples and Paraffin baiting technique for gelatine coated face mask but there was no growth in culture.

4. Discussion

The present study included 145 cases out of which there were 121 cases of progressive pulmonary disease caused by non - tuberculous mycobacteria which were diagnosed by culture method in microbiology department and 24 cases were diagnosed using sampling from gelatine coated face mask. Out of 121 cases in culture group, 82 were male and

39 were in females. In gelatine face mask group, 17 were males and 7 were females.

Out of 121 cases of NTM culture group, majority 39 (32.20%) were in age group of 15 - 40 years followed by 36 (29.80%) in age group of 41 - 60 years. Mean age was 51.10 ±18.28 years ranging from 15 - 85 years. In gelatine mask group, out of 24 cases, majority 14 (58.30%) were in age group of ≥61 years followed 06 (25%) in age group of 41 - 60 years. Mean age was 57.70±19.55 years ranging from 15 - 85 years. Sharma et al[12] in their study on NTM observed > 55 years (25.7%) followed by 46 to 55 years (22.86%) age group respectively. These findings were in contrast with present study as in culture group we had more cases in 15 - 40 years age group. Kapata et al[13] from Zambia found majority of cases (28%) in age group of >65 years followed by middle age group from 25 - 44 years which is in contrast with present study findings.

Out of 121 cases in culture group, maximum 82 (67.80%) were males and rest 39 (32.20%). In gelatine mask group, 17 (70.80%) were males and rest 07 (29.20%) were females.

In our study, majority 100 (82.64%) and 21 (87.50%) of cases in both the groups had breathlessness followed by cough in 83 (68.60%) cases and 16 (66.67%) cases respectively in culture and gelatine mask group. This was followed by fever in 77 (63.64%) culture group and 13 (54.17%) in gelatine mask group. Simons et al[14] in their profiling found chronic cough (255/268), followed by hemoptysis (82/268), fever (47/268), and weight loss (40/268) in their cases and Kapata et al[13] observed chest pain (71.07%) as most common clinical symptom followed by cough (52.98%) and fever (35.10%) which is comparable to present study findings.

In present study, out of 121 cases of culture group, 117 (96.70%) were negative for HIV and 04 (3.30%) cases had positive status of HIV while in gelatine mask group, all 24 cases were negative for HIV. In a study by Williams et al[15] on face mask Sampling for mycobacterium found one case of HIV positive status.

Majority cases had no history of tuberculosis in both groups respectively as 97 (80.20%) and 20 (83.30%). There were 24 (19.80%) cases in culture group and 04 (16.70%) cases in gelatin mask group with previous history of tuberculosis. Simons et al[14] observed 37% cases with previous history of tuberculosis which is higher than present study findings. Lim et al[16] mentioned 0.6% of cases with previous history of active tuberculosis.

In present study, in culture group, majority 64 (52.90%) had infiltrates followed by 48 (39.67.70%) cases had consolidation followed by 46 (38%), cases had tuberculosis Studies Positive HIV status (%) Simons et al[14] (2011) 5% Kapata et al [13] (2015) 5.85% Sharma et al[12] (2020) 2.8% Karamat et al[17] (2021)

5. Limitations

We missed large number of patients due to COVID positivity as they were sharing the same symptomatology. It was a single hospital based study and there may be diagnostic variabilities among different hospitals, because of its cross - sectional nature temporal associations could not be studied. Since the study was hospital based it may have omitted a certain spectrum of disease limiting its generalization.

6. Conclusion

In present study we could not be able to grow a single NTM from the study subjects. Diagnosis of NTM in the clinical specimen is often misleading and underestimates the incidence of NTM. Better diagnostic tools are needed as the currently available equipment for the diagnosis, species identification, and drug sensitivity testing of NTM are not fully developed.

7. Recommendations

In present study we could not grow a single NTM in present sample size. With decreasing prevalence of pulmonary TB and increasing Multi - drug resistant TB we have to suspect NTM infection if patient is not responding to usual line of management. Early suspicion and diagnosis of NTM will help the physician to reduce the time required for further management as the treatment is different for NTM. It will also help in reducing the TB burden of the community. A community based study with larger sample size including patients from different areas with varying symptoms could help achieve a more accurate result.

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Conflict of interest: None.

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