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Cross-sectional Evaluation of Mycobacterial Associations with Lung Disease and Its Associated Factors

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ABSTRACT

Background: Nontuberculous mycobacteria (NTM), a diverse group of environmental organisms rapidly proliferating in water, soil, and dust, are becoming a common cause of clinical disease. This study analyzed patient data from two major hospitals in Faisalabad, Pakistan, to improve early detection of NTM lung disease and to guide clinical practice in seeking earlier and quicker intervention.

Methods: A retrospective cross-sectional study was conducted from January 2020 to December 2021, using the records of 294 tuberculosis patients at Allied Hospital and DHQ Hospital, Faisalabad. Non-probability convenience sampling was used for sample collection and sample size was collected using OpenEpi 3.0.0. Data from patients with NTM lung disease were checked. The diagnosis was based on criteria defined by the ATS/IDSA (a clinical, radiological and microbiological evidence). Testing of specimens (sputum, BAL fluid, puncture fluid) was conducted using AFB smear, culture (MGIT 960) and species identification by molecular techniques. Chi-square, Wilcoxon tests and logistic regression were performed using SPSS version 26.0.$P<0.05$ was considered as significant.

Results: There were 294 patients (147 males; 147 females); median age 61 years, 77.2% had bronchiectasis. The most frequently identified species was the Mycobacterium avium-intracellulare complex (MAC 56.1%) followed by M. kansasii (19%) and M. abscessus (15.3%). Sputum cultures had the highest positivity rate (87.4%), outperforming BAL fluid (80.3%) and puncture fluid (61.5%).

Conclusion: The M. avium-intracellulare complex is the most common NTM species found in patients in these hospitals. The signs of expectoration, gender, and bronchiectasis increased the likelihood of BAL culture positivity, which aids in diagnosis.

Keywords: Nontuberculous Mycobacteria, Mycobacterium Tuberculosis, Bronchiectasis, Chronic Cough, Sputum, Epidemiology.

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INTRODUCTION

Nontuberculous mycobacteria (NTM), a diverse group of environmental organisms rapidly proliferating in water, soil, and dust, are becoming a common cause of clinical disease [1]. NTMs are not usually transmitted from person to person, and unlike Mycobacterium tuberculosis, can cause chronic pulmonary infections in people with underlying lung disease or with compromised immune systems [2,3]. NTM lung disease is becoming an increasingly recognized and significant public health issue, both in terms of its prevalence, diagnostic complexity, and cases of resistance to standard anti-tuberculous treatment regimens [4].

NTM lung disease poses special challenges for diagnosis. The clinical presentation often mimics tuberculosis (TB), with symptoms of chronic cough, sputum production, fatigue, and weight loss [5,6]. Frequently, this overlap leads to misdiagnosis or delays in instituting proper treatment. In addition, routine tools for TB diagnosis may not be successful in distinguishing the NTM infections, and specialized microbiological and molecular techniques are needed for accurate species diagnosis [7]. Diagnosis is guided by criteria established by the American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA), which include clinical, radiological, and microbiological components. Adherence to these criteria and access to these standards can vary widely in resource-limited settings [8].

Therefore, epidemiology and diagnostic patterns of NTM lung disease must be fully understood in order to improve patient outcomes. The diagnostic yield and time to treatment are highly influenced by factors such as the type of specimen tested, the NTM species assessed, and patient characteristics, including gender and concurrent lung diseases such as bronchiectasis [9].

Diverse causes of NTM lung disease and time to obtain a definitive diagnosis were assessed, along with factors associated with diagnostic delay or clinical outcomes, in this study. This study analyzed patient data from two major hospitals in Faisalabad, Pakistan, to improve early detection of NTM lung disease and to guide clinical practice in seeking earlier and quicker intervention.

METHODS

To determine the etiological spectrum and diagnostic patterns of NTM lung disease, a retrospective cross-sectional study was conducted from January 2020 to December 2021 at Allied Hospital and DHQ Hospital, Faisalabad. A total of 294 patients who met the inclusion criteria were analyzed. All adult patients (≥18 years) with bacteriologically confirmed NTM lung disease were included. Diagnosis was performed according to the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) criteria, based on clinical symptoms, radiographic evidence, and microbiological confirmation. Those subjects with incomplete clinical or laboratory data, or with associated Mycobacterium tuberculosis co-infection, were excluded.

The sample size was determined using OpenEpi version 3.0.0 (released 2013, Atlanta, GA, USA) [10]. Institutional protocols were followed for written informed consent from all patients at the time of diagnosis and data collection. For this study, the Institutional Review Board (IRB) of Punjab Medical College, Faisalabad granted ethical approval (9315-25). From the hospital tuberculosis surveillance database, patient demographics, clinical and smoking characteristics (cough, sputum, hemoptysis), comorbidities (e.g., bronchiectasis), radiologic findings, and laboratory data were retrieved. Sputum, bronchoalveolar lavage fluid (BALF), or puncture fluid were specified as the types of specimens analyzed. Molecular methods for species-level identification were performed on acid-fast bacilli (AFB) smear microscopy, culture for each sample.

The BACTEC MGIT 960 system was used for cultures. Speciation of positive isolates was performed using reverse hybridization line probe assays (Genotype NTM DR) or DNA sequencing. Different specimen types were compared on culture positivity rates. Statistical analysis was performed using SPSS version 26.0 (IBM Corp., Armonk, NY). Continuous variables were reported as medians with interquartile ranges, and the frequencies and percentages for categorical data. Associations between categorical variables were tested using the Chi-square test. When paired samples (e.g., culture vs. smear) were compared, Wilcoxon signed rank tests or McNemar's tests were employed. Binary logistic regression models were used to find predictors for culture positivity and diagnostic delays. Adjusted odds ratios (aOR; 95% CI) were calculated. A p value of less than 0.05 was considered statistically significant. Additionally, patient gender, bronchiectasis and culture positivity was used to test for associations using regression analysis.

This methodological approach provided a comprehensive evaluation of clinical and bacteriological patterns found in NTM lung disease, to assist reporting of diagnostic strategies and risk-based management of NTM disease in high-burden TB care settings.

RESULTS

Table 1: Demographic and Clinical Characteristics of NTM Patients

Variable	n (%)
Total Patients	294 (100%)
Male	147 (50%)
Female	147 (50%)
Median Age (IQR)	61 (46–69)
With Bronchiectasis	227 (77.2%)

n = Number of patients; IQR = Interquartile Range; % = Percentage; P = Probability value; Chi-square test

This cross-sectional study included 294 patients who had been diagnosed with NTM lung disease. The distribution of males and females was equal, and the median age of the study population was 61 years. More than three-fourths of the patients had bronchiectasis as the most common clinical feature. The predominant NTM species identified was Avium-intracellulare complex. Culture positivity rates were dependent on specimen type and clinical factors. **Table 1** lists the basic information and medical history of the NTM patients included in this study.

Table 2: Distribution of NTM Species and Culture Results by Sample Type

Variable	Value n (%)
Avium-intracellulare complex	165 (56.1%)
Mycobacterium kansasii	56 (19.0%)
Mycobacterium abscessus	45 (15.3%)
Other species (e.g., *M. malmoense*, *M. fortuitum*)	9 (3.1%)
Sputum Culture Positive	257 (87.4%)
BAL fluid Culture Positive	74 (80.3%)
Puncture Fluid Culture Positive	16 (61.5%)
Smear Positive Rate (Sputum)	142 (48.4%)
Stat Test (Smear vs Culture)	P < 0.01

NTM = Nontuberculous Mycobacteria; BAL = Bronchoalveolar Lavage; BALF = Bronchoalveolar Lavage Fluid; % = Percentage; P = Probability value

Out of 294 NTM patients, 50% were males and 50% were females. The median age was 61 years (interquartile range: 46–69 years). 77.2% of the patient cohort had bronchiectasis. Age distribution and prevalence of bronchiectasis were not significantly different between genders (Chi-square, P > 0.05). Table 2 describes how NTM species and culture results varied by the type of sample shown in **Table 2**.

Table 3: Logistic Regression Analysis of Diagnostic Delay Factors

Factor	aOR (95% CI)	P-value
Expectoration	0.48 (0.29–0.80)	<0.05
Cough	4.04 (1.80–9.05)	<0.001
Female Gender (BALF)	2.82 (1.16–6.88)	<0.05

Bronchiectasis (BALF)	2.38 (1.01–5.63)	<0.05
Mycobacterium abscessus (vs MAC)	0.43 (0.21–0.88)	<0.05
Rare NTM species (vs MAC)	8.31 (1.01–68.6)	<0.05

aOR = Adjusted Odds Ratio; CI = Confidence Interval; BALF = Bronchoalveolar Lavage Fluid; MAC = *Mycobacterium avium-intracellulare* complex; NTM = Nontuberculous Mycobacteria; vs = Versus; P = Probability value

Of NTM isolates, 56.1% were due to *Mycobacterium avium-intracellulare* complex. The sputum cultures had the highest positivity rate at 87.4%, culture of BALF (80.3%), and puncture fluid (61.5%). Smear positivity was significantly lower than that of culture (48.4% vs. 87.1%; $P < 0.01$). No statistical difference was observed between sputum and BALF culture positivity ($P > 0.05$). The findings from logistic regression analysis on diagnostic delay in NTM patients are shown in **Table 3**.

Those with expectoration had shorter diagnosis times (aOR = 0.48, 95% CI: 0.29–0.80). Sputum culture positivity was more than four times (aOR=4.04) likely in participants who had a cough. It was demonstrated that bronchiectasis and female gender influenced BALF culture positivity. Rare NTM species led to significantly increased diagnosis delays (aOR = 8.31, 95% CI: 1.01–68.6). Females and patients with bronchiectasis had significantly higher odds of BALF culture positivity (aOR = 2.82 and 2.38, respectively).

DISCUSSIONT

This cross-sectional study evaluated the microbiological and clinical profile of patients having NTM lung disease in two major hospitals. Results helped elucidate the species distribution, challenges with diagnostics and clinical features associated with positive cultures and diagnostic timelines. Mycobacterium avium-intracellulare complex was particularly interesting as we observed it comprising the predominant species. Expectoration and cough were significantly linked to higher sputum culture positivity and faster diagnosis; a major clinical feature was bronchiectasis. In contrast, significant diagnostic delays were associated with the rarer NTM species.

MAC predominance merely reflects global trends seen in this complex, which is known to be the most common etiological agent of NTM lung infections worldwide in patients with structural lung lesions or those who are immunocompromised [11]. As reported previously, bronchiectasis is observed more often in this population, providing further evidence for a cyclical relationship between NTM infection and bronchial wall damage [12]. Impaired mucociliary clearance and chronic airway inflammation resulting from bronchiectasis provide a basis for NTM colonization [13].

Diagnostic performance differed with sample types and it was demonstrated that sputum culture continues to be the most reliable and noninvasive method for NTM diagnosis, with culture positivity exceeding that of BAL and puncture fluid [14]. While the detection rate of smear microscopy was lower than that of culture, smear microscopy is still essential for initial screening, especially in resource-limited settings [15]. These results confirmed international guidelines which advise that smear should not be used to guide the diagnosis or management of NTM lung disease, but rather culture should be relied upon [16].

The role of cough and expectoration as clinical indicators of greater bacterial load or active infection was supported by the finding that cough and expectoration significantly increase the odds of positive sputum cultures [17]. These are consistent with the already recognized association of female gender and bronchiectasis with increased BAL culture positivity, which reflects a mode of presentation in women termed Lady Windermere syndrome [18,19]. It has been posited that those hormonal effects, immunological differences, and higher prevalence of CFTR gene mutations or connective tissue disorders explain why significantly more women are diagnosed with NTM pulmonary disease [20,21].

This study also brought to light a markedly challenging issue of diagnostic delay with rare NTM species. These species tend to present with non-specific symptoms and may necessitate more advanced diagnostic tools, which are not always available in all clinical laboratories [22]. Delayed diagnosis may lead to inappropriate treatment (such as empirical anti-TB therapy) and prolonged morbidity [23]. As a result, local laboratory capacity for molecular diagnostics and species-level identification is critical to effectively and efficiently manage and respond to such a situation [24,25].

One of the limitations of this study is that hospital records were used to obtain the data and may not have the full complement of clinical or diagnostic variables collected in all cases. The study was also conducted in two tertiary hospitals, so, results cannot be generalized to other regions and primary care settings. The second shortcoming is the absence of data seen later on what treatment outcomes or resistance patterns look like. Multi-center prospective studies with long-term follow-up and molecular resistance profiling should be part of future research in this population, which should address other potential genetic and environmental risk factors of disease development.

CONCLUSION

Mycobacterium avium-intracellulare complex is the predominant cause of NTM lung disease and bronchiectasis, along with clinical symptoms of cough and expectoration. This markedly enhances the diagnostic yield. Diagnosis is based on sputum culture, which remains the best diagnostic means; extremely rare NTM species can delay diagnosis. These findings highlight an unmet need for improved molecular diagnostics and targeted clinical awareness in order to allow for earlier identification and management of NTM lung infections.

LIST OF ABBREVIATIONS

NTM: Nontuberculous Mycobacteria
TB: Tuberculosis
IDSA: Infectious Diseases Society of America
ATS: American Thoracic Society

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CONFLICT OF INTEREST

None

ETHICAL APPROVAL

The ethical approval from the Institutional Review Board was taken under Allied Hospital-II, Faisalabad, under 9315-25.

AUTHORS' CONTRIBUTION

All contributed equally as per ICMJE.

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