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Clinical correlation study of non-tuberculous mycobacterial isolates from bronchoalveolar lavage fluid

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Abstract

Non-tuberculous mycobacterial (NTM) infections have emerged as a significant public health concern, posing a threat to human health. This study aims to identify various NTM strains from bronchoalveolar lavage fluid, assess their drug resistance profiles, and investigate the risk factors associated with NTM disease. Gene chip technology was used to identify NTM strains. The broth microdilution method assessed the drug sensitivity of isolated NTM pathogenic bacteria, determining their minimum inhibitory concentrations (MICs). Logistic regression analysis identified potential risk factors for NTM disease. Results showed the slow-growing NTM strains isolated from bronchoalveolar lavage fluid to be predominantly *Mycobacterium avium* and *Mycobacterium intracellulare*, accounting for 32.05% and 29.49% of the isolates, respectively. The rapidly growing NTM strains were mainly *Mycobacterium chelonae* and *Mycobacterium abscessus*, each constituting 25.64% of the isolates. *Mycobacterium avium* was found to be sensitive to clarithromycin, while linezolid demonstrated high antibacterial efficacy against *Mycobacterium intracellulare*. In drug susceptibility testing of *Mycobacterium chelonae* and *Mycobacterium abscessus*, amikacin exhibited the highest sensitivity, followed by clarithromycin. For patients with NTM-positive cultures, the risk factors for NTM lung disease included age (45–60 years, >60 years), a smoking history exceeding 10 years, chronic obstructive pulmonary disease (COPD), bronchiectasis, immunocompromised status, and the presence of thin-walled pulmonary cavities. Collectively, this study elucidates the distribution of NTM strains, their drug susceptibility profiles, and key risk factors for NTM lung disease, highlighting the need for proactive screening, early intervention, and targeted preventive strategies to improve diagnosis and optimize treatment outcomes.

Keywords Non-tuberculous mycobacteria, Infection, Tuberculosis, Lungs, Risk factors

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Introduction

Non-tuberculous mycobacterial (NTM) lung diseases have garnered increasing attention in recent years. While most NTM are saprophytic, only a subset is pathogenic. These pathogenic NTM can invade various tissues and organs, including the lungs, lymph nodes, bones, joints, skin, and soft tissues, potentially leading to systemic disseminated disease. More than 90% of NTM-related lung diseases are caused by pathogenic NTM (Dartois and Dick 2024). Recently, there has been a rapid rise in NTM disease, particularly among patients with clinically positive sputum acid-fast bacilli smears who are diagnosed with NTM lung disease. As a result, NTM disease has emerged as a major public health concern (Santos et al. 2024). The incidence of NTM disease has been increasing annually, yet the predominant pathogenic NTM species and their distribution characteristics vary by region, and the manifestations of NTM lung disease are evolving (Santos et al. 2024; Lim et al. 2018). In countries with a high burden of tuberculosis, such as China, NTM lung disease predominantly affects individuals with pre-existing chronic lung conditions (e.g., chronic bronchitis, bronchiectasis) (Larsson et al. 2017; Chu et al. 2024). Approximately one-third of AIDS patients may develop secondary NTM disease, with disseminated NTM disease commonly occurring in those with compromised immune function (Cuervo 2011; Mina et al. 2024). Without precise bacterial species identification, these patients might be misdiagnosed with tuberculosis.

Clinicians frequently encounter patients with positive sputum smears for acid-fast bacilli and corresponding clinical manifestations and imaging changes. These patients are often diagnosed with smear-positive pulmonary tuberculosis and receive anti-tuberculosis treatment, which may be ineffective for some, leading to persistent sputum positivity (Xu et al. 2024). In addition to evaluating factors such as treatment plan efficacy, patient compliance, and drug resistance, the possibility of NTM lung disease should also be considered, as most NTM are resistant to commonly used anti-tuberculosis drugs (e.g., streptomycin, ethambutol) and may require different clinical treatment approaches (Gopalaswamy et al. 2021). Thus, accurate identification of NTM is crucial for effective treatment. Current diagnostic technologies for NTM are lagging. Traditional bacterial species identification methods, which rely on biochemistry and differential culture media, are time-consuming and often fail to accurately identify many NTM species, leading to increased false positive and false negative rates (Zhang et al. 2024). Since no genetic identification method can fully identify NTM to the species level, clinical identification often necessitates a combination of methods (Zhang et al. 2024). Therefore, typing of identified NTM strains, when feasible, is important.

Once NTM disease is clinically confirmed, routine antimycobacterial drug susceptibility testing (DST) may no longer be necessary. Given that most NTM are resistant to common antimycobacterial drugs (Saxena et al. 2021). and the clinical relevance of DST results remains debated, DST is still important for establishing individualized treatment plans due to the variability in resistance patterns among different NTM species (Kamada and Mitarai 2021). There is ongoing controversy regarding the treatment of NTM lung disease, primarily due to uncertainties in treatment efficacy, high costs, and potential adverse reactions, which often result in an imbalance between investment and benefit (Philley et al. 2016). Therefore, not all NTM lung diseases require treatment, and an individualized treatment plan should be carefully considered.

Many hospitals in China and across the world face challenges in identifying NTM, leading to frequent clinical misdiagnoses (Dong et al. 2020). Although NTM isolation is increasingly common, its clinical relevance warrants further investigation. This study aims to identify mycobacterial species, examine NTM isolation and species distribution, analyse risk factors associated with NTM lung disease, and enhance prevention and control measures. Additionally, this study will conduct drug susceptibility tests on NTM strains to elucidate resistance patterns and guide the development of rational drug use plans in clinical settings.

Materials and methods

Patient information

Between May 2021 and May 2023, a total of 200 patients underwent bronchoscopy, either as inpatients or outpatients, with alveolar lavage fluid subsequently sent for mycobacterial culture. Patient data collected included general information such as age, gender, place of residence, and smoking history. Additional clinical history was recorded, including chronic lung diseases (e.g., chronic bronchitis, bronchiectasis, bronchial asthma, and chronic obstructive pulmonary disease (COPD), pneumoconiosis, chronic cough, recurrent hemoptysis, pulmonary cavities, and low immunity (e.g., history of anti-tuberculosis treatment, use of immunosuppressants, etc.). Prior informed written consent was sought from the patients for participation in the study. The study was approved by the ethics committee of Wenzhou Central Hospital, Dingli Clinical College of Wenzhou Medical University, Wenzhou, Zhejiang, China (Approval number: WCH0061721).

Inclusion criteria

The inclusion criteria for this study required that patients exhibit clinical manifestations such as a cough and sputum production lasting more than two weeks,

accompanied by symptoms like hemoptysis, chest pain, low-grade afternoon fever, night sweats, and fatigue. Imaging findings needed to show one or more of the following: cavitary lesions, tree-in-bud signs, exudation, proliferation, caseous lesions, or associated pleural effusion or thickening. Additionally, patients must present with a pulmonary infection that is difficult to differentiate from tuberculosis. For suspected NTM lung disease, patients should have risk factors for low immunity, persistent or recurrent symptoms, and sputum positive for acid-fast bacilli, with primary symptoms focused on chronic respiratory issues rather than significant tuberculosis poisoning. Chest imaging should reveal bronchiectasis in the middle lobe and lingual segment of the lung, multiple centrilobular nodules, tree-in-bud signs, and thin-walled cavities in the upper lobes, possibly accompanied by less dense infiltration around the cavity walls and lung lesions characterized by fibroproliferative changes. Furthermore, the PPD test should be weakly positive, and there should be a documented response to formal anti-tuberculosis treatment, including initial treatment, re-treatment, and multi-drug-resistant tuberculosis.

Exclusion criteria

The exclusion criteria for this study included patients with severe hepatic or renal insufficiency, advanced heart failure, or severe systemic infections. Individuals in the acute phase of cerebral infarction were also excluded, as were those diagnosed with malignant neoplasms. These criteria were established to ensure that the study focused on a population where NTM lung disease could be accurately assessed without the confounding effects of these serious medical conditions.

Acid-fast bacilli smear and BACTEC-MGIT960 culture

Sputum or alveolar lavage fluid samples were centrifuged and concentrated prior to smearing. The BACTEC-MGIT960 liquid culture system was utilized for rapid culture, and an acid-fast bacilli smear was performed to confirm the presence of mycobacteria following a positive mycobacterial culture.

Rapid primary screening and identification of Mycobacteria

Initial identification of *Mycobacterium tuberculosis* or non-tuberculous mycobacteria (NTM) was conducted by measuring the MPB64 protein, a mycobacterial protein derived from *Mycobacterium bovis* BCG, during culture.

NTM strain identification

Identification of NTM strains was performed using the gene chip method as described previously (Liu and Ma 2019). Briefly, positive strains from modified Roche medium were selected, and bacterial concentration was adjusted to approximately 1 McFarland unit. Twenty microliters of bacterial solution were combined with 80 μL of nucleic acid lysis solution, mixed thoroughly, and incubated at 100 °C for 10 min for complete lysis. The mixture was then centrifuged at 12,000 rpm for 1 min, and the supernatant was used as the DNA template. Eighteen microliters of the amplification reagent were added to an eight-strip tube. Two microliters of the DNA template were added, and the mixture was subjected to amplification using the following conditions: 37 °C for 600 s, 94 °C for 600 s, followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s. An additional 10 cycles included 94 °C for 30 s, 72 °C for 60 s, with a final extension at 72 °C for 420 s. The amplified product was denatured at 95 °C for 5 min, then rapidly cooled at -20 °C for 5 min. During hybridization, 9 μL of hybridization buffer was mixed with 6 μL of the gene amplification product. Thirteen and a half microliters of the mixed solution were applied to the chip, which was then placed in a hybridizer at 50 °C for 2 h. Post-hybridization, the chip was washed, dried, scanned, and analyzed.

Drug sensitivity testing

Drug susceptibility testing was performed using the broth microdilution method. Briefly, the bacterial inhibitor, drug susceptibility culture medium, and 96-well drug susceptibility test plate were equilibrated to room temperature. Two hundred microliters of bacterial inhibitor were added to the drug susceptibility culture medium and thoroughly mixed. One hundred eighty microliters of the drug susceptibility culture medium were added to wells E1 and F1 as 1/10 and 1/100 reference wells, respectively. Two hundred microliters of the medium were added to wells A1 and B1 as negative controls. Using an ultrasonic dispersion instrument, the bacterial concentration was adjusted to 1 mg/mL. Two hundred microliters of this bacterial suspension were added to the entire volume of drug susceptibility culture medium and mixed well. Two hundred microliters were transferred from each well (excluding the four reference wells) into the remaining wells. Twenty microliters of the bacterial culture medium were drawn from well E1 to create a 1/10 reference well, and 20 μL from well E1 to well F1 for a 1/100 reference well. The plate was covered, sealed with transparent tape, and incubated at 37 °C.

Observation indicators

Results were assessed on the third day post-incubation. Positive control wells (E1 and F1) were expected to show white precipitate at the bottom, whereas negative control wells (A1 and B1) should not exhibit growth. The minimum inhibitory concentration (MIC) value for each drug was recorded as the lowest drug concentration without

Table 1 Clinical and demographic characteristics of NTM patients

Index	NTM patient ($n=200$)
Age	
Mean age	55.52 ± 9.65 years
< 45 years	43 (21.5%)
45–60 years	101 (50.5%)
> 60 years	68 (34%)
Gender	
Male	107 (53.5%)
Female	93 (46.5%)
Smoking history	
None	71 (35.5%)
< 10 years	50 (25%)
10–20 years	45 (22.5%)
> 20 years	34 (17%)
Place of residence	
Urban	110 (55%)
Rural	90 (45%)
COPD	54 (27%)
Bronchiectasis	95 (47.5%)
Pneumoconiosis	21 (10.5%)
Immunocompromised	16 (8%)
Chronic cough	172 (86%)
Repeated hemoptysis	84 (42%)
Cavities in the lungs	
None	112 (56%)
Thin walled	53 (26.5%)
Thick walled	35 (17.5%)

white precipitate. If growth was not observed in the positive control wells, further culturing was performed, with observations every 1 to 2 days until 21 days. Drug resistance breakpoints were determined according to CLSI standards.

Statistical analysis

The data were analyzed using SPSS 21.0 and entered and verified with EPIDATA software. Continuous normally distributed data are expressed as mean ± standard deviation ($\bar{x} \pm s$) and analyzed using an independent sample *t*-test, while multiple group comparisons were conducted using analysis of variance (ANOVA) with Dunnett's post-hoc test. Non-normally distributed data were assessed with the non-parametric Mann-Whitney *U* test. Logistic regression analysis was performed to identify risk factors.

Results

Comparison of clinical data

An analysis of the clinical characteristics of the patient cohort was conducted, focusing on factors associated with non-tuberculous mycobacterial (NTM) lung disease and their respective classification methods (Table 1). The findings indicate that a significant proportion of patients, specifically 47.5%, were affected by bronchiectasis.

Table 2 Distribution of NTM species isolated from patients

Species	Cases	Constituent ratio (%)
Mycobacterium avium	25	32.05
Mycobacterium intracellulare	23	29.49
Mycobacterium chelonae and abscessus	20	25.64
Mycobacterium gordonii	5	6.41
Mycobacterium kansasii	3	3.85
Mycobacterium xenopus	2	2.56

Chronic cough was reported by 86% of the patients, while recurrent hemoptysis was noted in 42% of the cases. Additionally, a history of smoking was observed in 64.5% of the individuals.

Identification of NTM species

The slow-growing mycobacteria identified in the randomly selected non-tuberculous mycobacteria (NTM) isolates were predominantly *Mycobacterium avium* and *Mycobacterium intracellulare*, accounting for 32.05% and 29.49% of the isolates, respectively. The fast-growing mycobacteria were primarily *Mycobacterium chelonae* and *Mycobacterium abscessus*, which collectively comprised 25.64% of the isolates. Additionally, a small number of mycobacterial species, including Mycobacterium gordonii and *Mycobacterium kansasii*, were isolated. Due to the limited number of these strains, they were excluded from the subsequent drug susceptibility analysis (Table 2; Fig. 1).

Drug susceptibility test results

Mycobacterium avium

Drug susceptibility testing was conducted using drugs with resistance breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI). The results are summarized in Table 3. *Mycobacterium avium* demonstrated 100% susceptibility to linezolid and amikacin. Sensitivity to rifampicin, clarithromycin, moxifloxacin, ethambutol, and rifabutin was above 80%. In contrast, resistance to doxycycline and minocycline exceeded 80%. These findings suggest that, for treating infections caused by *Mycobacterium avium*, selecting drugs to which the organism is sensitive would be appropriate.

Mycobacterium intracellulare

Susceptibility testing for *Mycobacterium intracellulare* was performed, and the results are presented in Table 4. The organism demonstrated high sensitivity to several drugs, with sensitivity rates exceeding 80% for linezolid, moxifloxacin, and amikacin. Sensitivity to rifabutin and clarithromycin was greater than 70%. In contrast, resistance to minocycline was found to be over 90%.

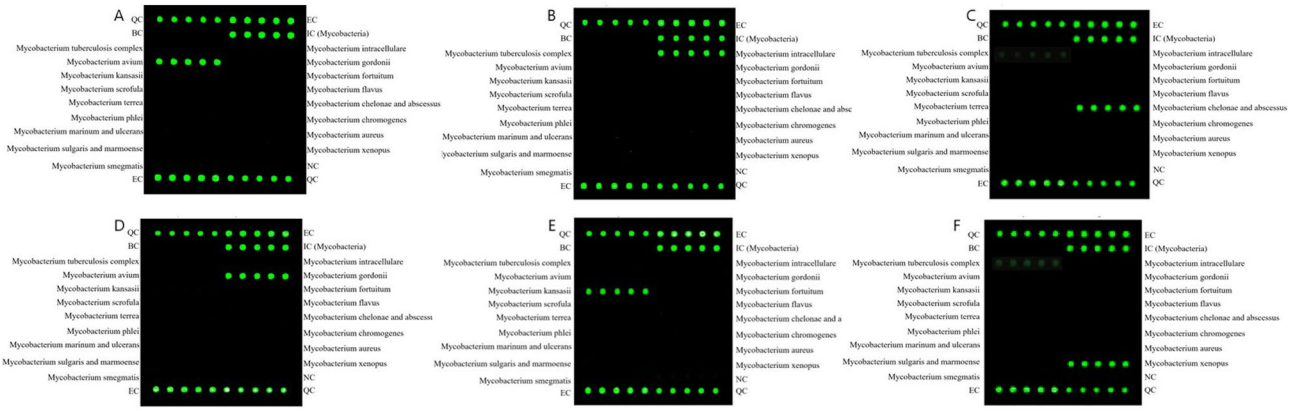

Fig. 1 Representative chart of NTM strain identification results **A***Mycobacterium avium*, **B***Mycobacterium intracellulare*, **C***Mycobacterium chelonae* and *Mycobacterium abscessus*, **D***Mycobacterium gordoni*, **E***Mycobacterium kansasii*, **F***Mycobacterium xenopus*

Table 3 Drug susceptibility test results for *Mycobacterium avium* (%, $n = 25$)

Drug	MIC range (μg/mL)	Sensitive ratio (%)	Moderately sensitive ratio (%)	Resistant ratio (%)
Rifampicin	1–16	86.5	-	13.5
Clarithromycin	0.5–64	93.5	6.7	0
Moxifloxacin	0.125–16	85.0	6.7	6.7
Doxycycline	0.5–128	6.7	0	93.3
Minocycline	0.5–128	6.7	13.3	80.0
Sulfamethoxazole	8–256	60.0	-	40.0
Linezolid	0.5–32	100.0	0	0
Amikacin	1–64	100.0	0	0
Ethambutol	2.5–20	80.0	6.7	13.4
Rifabutin	0.5–32	93.5	-	6.7

Table 4 Drug susceptibility test results for *Mycobacterium intracellulare* ($n = 23$)

Drug	MIC range (μg/mL)	Sensitive ratio (%)	Moderately sensitive ratio (%)	Resistant ratio (%)
Rifampicin	1–16	70.0	-	30.0
Clarithromycin	0.5–64	75.0	0	25.0
Moxifloxacin	0.125–16	85.0	5.0	10.0
Doxycycline	0.5–128	0.0	10.0	90.0
Minocycline	0.5–128	10.0	0	90.0
Sulfamethoxazole	8–256	40.0	-	60.0
Linezolid	0.5–32	85.0	0	15.0
Amikacin	1–64	95.0	0	5.0
Ethambutol	2.5–20	50.0	0	50.0
Rifabutin	0.5–32	75.0	-	25.0

Mycobacterium chelonae and Mycobacterium abscessus

Susceptibility testing was conducted for *Mycobacterium chelonae* and *Mycobacterium abscessus*. The results indicated that these exhibited 93.7% sensitivity to amikacin and 62.5% sensitivity to clarithromycin. The resistance rates were found to be over 93% for the drugs such as minocycline, and tobramycin across the strains (Table 5).

Table 5 Drug susceptibility test results for *Mycobacterium chelonae* and *Mycobacterium abscessus* ($n = 20$)

Drug	MIC range (μg/mL)	Sensitive ratio (%)	Moderately sensitive ratio (%)	Resistant ratio (%)
Amikacin	1–16	93.7	0.0	6.3
Cefoxitin	4–160	31.3	43.7	25.0
Clarithromycin	0.5–64	62.5	6.3	31.2
Doxycycline	0.5–128	0.0	0.0	100.0
Minocycline	0.5–128	0.0	0.0	100.0
Linezolid	0.5–32	62.5	0.0	37.5
Moxifloxacin	0.125–16	6.3	25.0	68.7
Tobramycin	0.5–64	6.3	0.0	93.7

Logistic regression analysis

An initial univariate analysis was performed to evaluate various variables associated with NTM lung disease, applying a significance threshold of $\alpha = 0.05$. Factors that emerged as significantly related ($p < 0.05$) included age (45–60 years old and >60 years old), smoking history (<10 years, 10–20 years, >20 years), COPD, bronchiectasis, pneumoconiosis, immunocompromised status, and the presence of thin-walled cavities in the lungs (Table 6). Subsequent to this preliminary screening, a multivariate logistic regression analysis was undertaken. The findings suggested that the identified risk factors for NTM lung disease encompassed age, smoking history, COPD, bronchiectasis, pneumoconiosis, immunocompromised status, and the presence of thin-walled cavities in the lungs (Table 7).

Table 6 Single factor regression analysis of risk factors for NTM lung disease

Index	β	*P*	OR	95% CI
Age				
45–60 years	0.853	< 0.001	2.346	1.637–4.258
> 60 years	1.396	< 0.001	4.038	2.573–6.234
Gender (male/female)	-1.228	0.881	0.293	0.837–1.096
Smoking				
< 10 years	-4.526	0.029	0.636	0.218–0.994
10–20 years	1.886	0.003	1.886	1.875–3.175
> 20 years	1.093	0.048	2.982	1.463–3.592
Place of residence				
Urban/rural	-4.510	0.115	0.637	0.364–1.117
COPD	0.074	0.016	1.077	1.376–2.011
Bronchiectasis	0.319	0.002	1.376	1.268–2.402
Pneumoconiosis	-2.984	0.041	0.742	1.298–1.847
Immunocompromised	-5.763	0.028	0.562	0.396–0.610
Chronic cough	0.482	0.247	1.619	0.716–3.659
Repeated hemoptysis	0.447	0.121	1.563	0.888–2.751
Cavities in the lungs				
Present	0.900	0.003	2.460	1.755–3.826
Absent	-2.523	0.519	0.777	0.362–1.670

Table 7 Multifactor regression analysis of risk factors for NTM lung disease

Index	β	*P*	OR	95% CI
Age	-0.046	0.006	1.05	0.923–0.987
Smoking	0.362	0.023	1.436	0.248–3.310
COPD	0.922	0.000	2.515	0.196–1.601
Bronchiectasis	0.053	0.006	1.054	0.593–0.754
Pneumoconiosis	-1.030	0.000	0.357	0.792–1.033
Immunocompromised	0.138	0.037	1.148	0.772–0.959
Cavities in the Lungs	1.234	0.003	3.436	1.792–6.441

Discussion

The rise in NTM isolation has introduced significant clinical uncertainty, with ongoing debates regarding whether these isolates represent colonization, contamination, or true NTM lung disease (Dhasmana et al. 2024). The clinical correlation between NTM isolates from respiratory specimens remains poorly studied both domestically and internationally, and there are notable regional variations in the prevalence and distribution of NTM lung diseases (Ratnatunga et al. 2020). Geographical differences in China reveal that the prevalence of NTM is higher in the southern regions compared to the north, along the coast compared to inland areas, and in mild climate regions compared to cold climates (Zhou et al. 2020). NTM are widespread in the environment and act as opportunistic pathogens. The mere isolation of NTM does not necessarily indicate pathogenicity, and contamination or environmental exposure must be ruled out (Ghio et al. 2019). NTM-related diseases predominantly affect the lungs, with approximately 90% of cases involving pulmonary manifestations. The species of NTM that are prevalent vary by country (Goswami et al. 2016; Park et al. 2017), and their pathogenicity differs accordingly. With rising NTM infections globally (Santos et al. 2024), understanding the clinical relevance of NTM isolates is essential for refining diagnostic protocols and preventing misdiagnosis as tuberculosis, which can lead to inappropriate treatment strategies. Epidemiological research on NTM diseases is challenging, as reporting is not mandatory in many countries, making it difficult to obtain accurate data for specific regions. Distinguishing between NTM infection and disease is also problematic, leading to variability in infection rates and prevalence across studies. Available data suggest that the incidence and prevalence of NTM infections are increasing in certain countries and regions, potentially surpassing those of tuberculosis (Lee et al. 2020; Schiff et al. 2019). For instance, *Mycobacterium xenopi* is noted to be more pathogenic in Canada and Europe compared to the United States, whereas *Mycobacterium intracellulare* and *Mycobacterium avium* exhibit the opposite trend in pathogenicity between the Netherlands and the United (Forbes et al. 2018). Understanding the resistance patterns of NTM is crucial for effective clinical management of NTM diseases. Currently, NTM drug susceptibility tests (DSTs) are not widely conducted in primary care laboratories, and the previous reporting models were based solely on *Mycobacterium tuberculosis* (van Ingen et al. 2012). Therefore, DSTs specific to NTM species are vital for developing appropriate treatment strategies. In this study, strains isolated and identified as NTM since May 2021 were characterized using a gene chip method. The primary focus of drug susceptibility testing (DST) was on Mycobacterium avium, *Mycobacterium intracellulare*, *Mycobacterium chelonae*, and *Mycobacterium abscessus*. Given that NTM infections often require long-term multidrug regimens, our findings emphasize the need to incorporate routine DST into clinical workflows to optimize antibiotic selection and reduce the risk of resistance development. For *Mycobacterium avium* infections, current guidelines recommend in vitro testing of clarithromycin and suggest combination therapy with at least two antibiotics, including clarithromycin (Hu 2016). Our findings indicate that *Mycobacterium avium* is relatively sensitive to clarithromycin, consistent with existing literature (Liu et al. 2019; Özdemir et al. 2020). This suggests that clarithromycin-based regimens should remain a primary treatment option for *M. avium* infections, with moxifloxacin and rifabutin as potential alternatives when resistance arises. Additionally, *Mycobacterium avium* showed sensitivity to rifampicin, moxifloxacin, linezolid, amikacin, and rifabutin. *Mycobacterium intracellulare* exhibited slightly reduced sensitivity to clarithromycin compared to *Mycobacterium avium*, but showed higher sensitivity to moxifloxacin, linezolid, and amikacin, with sensitivities to rifampicin and rifabutin also exceeding 70%. Notably, this study observed

that linezolid has high antibacterial efficacy against *Mycobacterium intracellulare* and is in agreement with a study a previous study (Hu 2016) but differ from another study (Liu et al. 2019). This discrepancy may be attributable to regional variations in treatment practices or resistance patterns. For *Mycobacterium chelonae* and *Mycobacterium abscessus*, amikacin demonstrated the highest sensitivity, followed by clarithromycin, which is consistent with recommendations in the "Expert Consensus on Diagnosis and Treatment of Non-Tuberculous Mycobacterial Diseases" (Tuberculosis Branch of the CMA 2012). Furthermore, this study found high resistance to doxycycline and minocycline, in line with previous reports (Litvinov et al. 2018) suggesting that these antibiotics may be unsuitable for treating NTM infections. Among patients with positive sputum smear/culture, only a small proportion are diagnosed with NTM lung disease. However, recent global reports indicate a notable increase in the incidence of NTM lung disease (Pedrero et al. 2019; Liu et al. 2021). The difficulty in distinguishing NTM lung disease from tuberculosis is compounded by the traditional 6–8 week culture period. Although newer molecular techniques, such as 16 S rRNA gene sequencing and PCR, offer quicker identification of NTM (Gopalaswamy et al. 2020) widespread clinical adoption remains limited. Misdiagnosis as tuberculosis often leads to inappropriate treatment with anti-tuberculosis drugs, to which NTM are generally resistant or can develop resistance easily (Chin et al. 2019; Gopalaswamy et al. 2020) resulting in poor treatment outcomes. Developing region-specific diagnostic guidelines incorporating rapid molecular testing for NTM detection could significantly enhance early diagnosis and treatment selection, minimizing misdiagnosis and unnecessary tuberculosis treatment. This study aims to explore the risk factors associated with NTM lung disease in patients with positive bacterial cultures to aid in the prevention and management of NTM lung disease. Studies have identified elderly women over 60 years of age in Canada as a significant risk factor for NTM lung disease, with their risk increasing approximately 2.3-fold (Hernandez-Garduno and Elwood 2010). Our study similarly identifies middle-aged and elderly individuals as risk factors, with a 2.6-fold increased risk for those aged 45 to 60 years and a four-fold increase for those over 60 years. Gender appears to have little correlation with NTM lung disease risk, which may be influenced by racial and regional differences. The respiratory tract is a major target for microbial infections. While NTM generally exhibit lower virulence compared to *Mycobacterium tuberculosis* and are less pathogenic, they act as opportunistic pathogens (Thibault et al. 2020). Literature suggests that in adults with normal immune function, NTM lung disease often occurs secondary to chronic underlying lung conditions with prior respiratory obstruction and/or structural damage, such as COPD, bronchiectasis, or pneumoconiosis, and is frequently accompanied by a significant smoking history (Ahmed et al. 2020).

Our study corroborates that COPD and bronchiectasis are significant risk factors for NTM lung disease, with COPD patients having approximately 2.5 times and bronchiectasis patients about 1.1 times increased risk. Given these findings, proactive screening for NTM in COPD and bronchiectasis patients could facilitate earlier detection and improve patient outcomes. This may be due to compromised mucosal barriers and impaired airway immunity in these patients, creating a conducive environment for NTM growth. Additionally, the presence of thin-walled cavities in the lungs significantly increases the risk of NTM lung disease, with a risk increase of about 3.4 times, consistent with findings that NTM lung disease often presents with such cavities (Chai et al. 2022; Pennington et al. 2021).

Despite interestingly results, our study has limitations. First, the lack of molecular investigations into the mechanisms underlying drug resistance in NTM strains limits our understanding of resistance evolution and potential therapeutic targets. Future studies should explore genetic mutations associated with antibiotic resistance in NTM species. Second, species-specific DSTs were not performed for all NTM strains, as certain strains were excluded based on clinical assumptions about their pathogenicity. Additionally, the study was conducted in a single hospital, which may limit its generalizability to other regions.

Future research should focus on developing standardized molecular diagnostic tools for rapid NTM identification, evaluating novel treatment regimens tailored to resistance patterns, and investigating host-pathogen interactions to identify potential therapeutic targets.

In conclusion, this study identified *M. avium* and *M. intracellulare* as predominant slow-growing NTM and *M. chelonae* and *M. abscessus* as the most common rapidly growing species. Drug susceptibility testing revealed high sensitivity of *M. avium* to clarithromycin, while amikacin was most effective against *M. chelonae* and *M. abscessus*. Key risk factors for NTM lung disease included age ≥ 45 years, smoking history, COPD, bronchiectasis, and immunocompromised status. Given resistance variability, species-specific drug susceptibility testing should guide treatment, and early molecular identification should be integrated into clinical practice. Future research should explore molecular mechanisms of drug resistance to improve therapeutic strategies.

Abbreviations

NTM	Non-tuberculous mycobacterial
COPD	Chronic obstructive pulmonary disease
DST	Drug susceptibility testing
TGF-β1	Transforming growth factor-β1

Author contributions
HN and XJ: conceived and designed the experiments. HN, GH, YM, JY, JS, XC and CQ: performed the experiments. HN, and GH: wrote the manuscript. XJ: polished the manuscript. All authors read and approved the manuscript.

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Data availability
Available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the ethics committee of the ethics committee of Wenzhou Central Hospital, Dingli Clinical College of Wenzhou Medical University, Wenzhou, Zhejiang, China (Approval number: WCH0061721). All patients provided informed consent.

Consent for publication
Not applicable.

Competing interests
The authors have no conflict of interest to declare.

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