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Original Article

Comparison of efficacies of different treatments for nontuberculous mycobacterial pulmonary disease in Anhui Province, China

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Abstract

Introduction: Although nontuberculous mycobacterial (NTM) infection is a common cause of pulmonary disease worldwide, few studies have focused on epidemiological and therapeutic factors related to NTM cases in Anhui Province, China. This retrospective study aimed to identify aetiological and clinical factors, and treatment outcomes of patients with NTM pulmonary disease (NTMPD) in Anhui.

Methodology: Retrospective clinical data obtained from medical records of NTMPD patients seeking care at Anhui Chest Hospital from July 2019 to June 2022 were analyzed. Treatment outcomes were compared between two patient groups: one receiving a standardised NTM treatment regimen and the other receiving precision treatment regimens.

Results: Genotypic analysis of 672 clinical NTMPD-associated isolates revealed that most were *Mycobacterium intracellulare*, while drug-susceptibility test results demonstrated diverse antibiotic resistance profiles for these isolates. Cough was the most common symptom for 101 NTMPD patients. After patients of both groups received treatment, symptoms improved, sputum culture conversion was observed for some patients, imaging findings stabilised; however, no statistically significant intergroup differences in treatment outcomes were found.

Conclusions: In this study, *M. intracellulare* was the predominant NTM species identified in isolates obtained from NTMPD patients. Drug resistance profiles of our patient isolates were complex, highlighting the need for administration of timely, more effective, standardised treatments for patients with NTMPD in Anhui Province, China.

Key words: nontuberculous mycobacterium; epidemiology; therapeutic.

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Introduction

Nontuberculous mycobacteria (NTM) are primarily environmental organisms and typically do not cause disease in humans. Nevertheless, certain NTM species are opportunistic pathogens, capable of illnesses distinct from tuberculosis (TB) and leprosy caused by *Mycobacterium tuberculosis* (MTB) and *Mycobacterium leprae*, respectively.

In recent years, more than 30 newly identified NTM species have been discovered, many of which exhibit heightened pathogenicity toward humans. An analysis of publicly available data revealed a strikingly high clinical NTM isolation rate in China. However, this rate likely underestimates the true clinical NTM prevalence rate, since pathogenic NTM are frequently unculturable. In cases where a NTM isolate cannot be cultured, diagnoses of patients presenting with pulmonary illnesses of unknown aetiology must rely on clinical findings by experienced physicians [1–3].

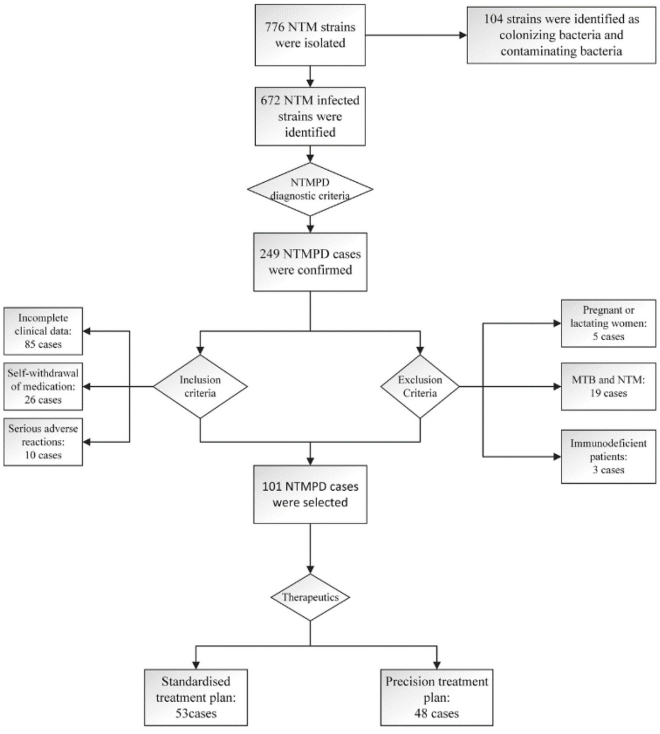
As opportunistic pathogens, NTM predominantly infect vulnerable individuals, including those with immunodeficiencies, histories of drug abuse, or exposures to specific environmental factors. Once inside susceptible hosts, NTM organisms can infiltrate various tissues and organs, such as lungs, lymph nodes, skin, and bones [4–6]. Unlike TB, which is caused by MTB, diseases caused by NTM are particularly difficult to treat, due to their inherent resistance to most antibiotics. Moreover, research studies have shown that even individuals who have been successfully treated for NTM disease remain susceptible to reinfection upon re-exposure to these opportunistic pathogens [7,8].

NTM pulmonary disease (NTMPD) is the most prevalent form of NTM-related disease, accounting for approximately 70–80% of reported cases outside of China. However, epidemiological survey data based on a large number of isolates obtained from Chinese NTMPD cases have not yet been reported. Nevertheless, findings of smaller NTMPD-focused Chinese studies have indicated a notable increase in the NTM isolation rate from 4.3% in 1979 to 22.9% in 2010, suggesting growing incidence of NTM disease in the country. Susceptibility to NTM infection is influenced by various host risk factors, including underlying pulmonary conditions, drug-induced or disease-induced immunodeficiencies, and environmental factors such as exposure to tap water [2].

Current guidelines for NTM treatment primarily rely on consensus clinical opinions or case reports rather than well-designed large clinical studies. Consequently, current treatments administered to NTMPD patients tend to mirror treatment regimens used for drug-resistant TB. However, these regimens often consist of multiple antibiotics that can potentially trigger serious adverse events. Furthermore, global NTMPD-associated mortality rates have not been determined, due to a lack of studies analyzing treatment outcomes and disease-related adverse events in NTMPD patients [3,9,10].

This study focuses on Anhui Province, a region in central China with a population of over 63 million people. Before 2018, Anhui reported few cases of NTMPD due to limited economic, technological, and healthcare resources there. Our study aimed to retrospectively analyze clinical characteristics of NTMPD patients in Anhui Province using available resources in that region. Moreover, we compared efficacies of standardised and precision NTMPD treatment regimens to provide insights and to guide the development of improved diagnosis and treatment strategies for use in managing NTMPD patient care in resource-limited areas such as Anhui Province.

Figure 1. The flow chart of selection of NTMPD patients.



NTM: nontuberculous mycobacterial infection; NTMPD: nontuberculous mycobacterial pulmonary disease.

Methodology

Study design and population

In this study, we retrospectively collected clinical data from medical records of NTMPD patients seeking care at Anhui Chest Hospital from July 2019 to June 2022. We also collected sputum, bronchoalveolar lavage fluid (BALF), and pus specimens from the patients whose post-culture yielded a total of 776 clinical isolates. Following completion of microbiological screening and identification assays, 104 isolates were identified as normal flora or environmental contaminants, leaving a total of 672 NTM isolates, including 668 cultured from sputum and BALF samples and four cultured from pus samples. Given the NTMPD focus of this study, we selected 101 NTMPD cases with complete clinical data for further statistical analysis. The patient selection process is outlined in Figure 1. Selected patients were assigned to two treatment groups based on NTM drug resistance profiles determined via drug susceptibility testing (DST): the standardised treatment group (53 cases) and the precision treatment group (48 cases).

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committees of Anhui Chest Hospital(K2021-013). Informed consent was obtained from all subjects involved in the study.

Laboratory testing methods: NTM culture, species identification, and drug susceptibility tests (DST)

All patient specimens, including sputum, BALF, and pus, were pretreated and cultured using the BACTEC MGIT 960 liquid culture method (Becton, Dickinson and Company, Maryland, USA). Isolates testing positive via the colloidal gold method with para-nitrobenzoic acid (PNB) medium (Baso Biotechnology

Co., Ltd., Zhuhai, China) were tentatively identified as NTM using an MPB64 antigen detection kit (Genesis Bio-detection & Biocontrol Co., Ltd., Hangzhou, China). Isolates preliminarily identified as NTM were then classified at the species level using the MeltPro Myco assay using a pan-mycobacterial primer set (Zeesan Biotech, Xiamen, China). Species assignments were conducted based on proteomic fingerprinting via matrix-assisted laser desorption ionisation−time of flight mass spectrometry (MALDI-TOF MS) and interpreted using the Bruker MALDI-TOF MS identifier (Agena Bioscience, Shanghai, China).

DST was performed following the broth microdilution method reported previously by Wang *et al.* [11]. Briefly, NTM cells were seeded into microplates containing serial dilutions of 15 antimicrobial drugs. DST results were read using a microplate reader (YK-909, Encode Medical Engineering Co., Ltd., Zhuhai, China). Tested antimicrobial drugs included amikacin, cefoxitin, clarithromycin, doxycycline, tobramycin, azithromycin, rifabutin, minocycline, linezolid, rifampicin, gatifloxacin, ethambutol, moxifloxacin, sulphamethoxazole, and imipenem-cilastatin. Interpretation of results was carried out in accordance with instructions provided with the microplate NTM DST kit (Intech Medical Engineering Co., LTD., Zhuhai, China). Quality control strains for DST included ATCC 927, ATCC 27853, and ATCC 29213.

Clinical data

Patient selection

Data were retrospectively collected from medical records of 101 NTMPD patients treated at Anhui Chest Hospital between July 2019 to June 2022. The patient cohort consisted of 53 males and 48 females with a male-to-female ratio of 1.1:1. The average age of patients was 59.8 (± 13.2) years, with ages ranging from 21-89 years. All patients received treatment for NTMPD for a minimum period of 9 months.

NTMPD patients were identified based on diagnostic criteria outlined in the British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD) (2017) [12] and the Chinese expert consensus on diagnosis and treatment of NTM diseases (2012) [1]. NTMPD diagnosis was based on exclusion of other diseases and relied on observed respiratory symptoms and/or systemic symptoms, along with chest imaging findings showing cavitary shadows, multifocal bronchiectasis, and/or multiple small nodular lesions. During collection of patient sputa, BALF, and pus specimens, precautions were taken to prevent contamination of specimens with exogenous organisms. Additionally, patients met one of the following criteria: (i) two positive sputum cultures for the same NTM species; (ii) BALF yielding one NTM-positive culture result and/or one NTM-positive molecular biological test result, along with a positive acid-fast bacilli smear score of ++ or higher; (iii) sputum yielding one NTM-positive culture result and/or BALF yielding one positive molecular biological test result, along with a positive acid-fast bacilli smear score of ++ or higher.

Patients excluded from the study included pregnant or lactating women, patients infected with both MTB and NTM, immunodeficient patients, and those who discontinued treatment for various reasons.

All patients included in the study also met the following criteria: (i) medical records contained complete clinical data, such as sputum acid-fast bacillar smear, sputum culture, chest computed tomography (CT) scan results, and other findings and test results; (ii) strictly adhered to physician-guided medication administration and refrained from altering or discontinuing treatments without physician approval; (iii) experienced no serious adverse reactions during treatment.

Treatment

The patients were assigned to two groups based on DST results, one receiving the standardised treatment and one receiving precision treatments. In the standardised treatment plan, patients received treatment with at least four different types of drugs following established guidelines and consensus recommendations. In the precision plan, patients initially received antibiotics similar to those in the standardised plan. However, once DST results were available, only drugs showing antimicrobial activities against their specific NTM isolates were continued or added to the regimen, while drugs lacking such activity were discontinued. The updated precision treatment plan included a minimum of five drugs with activity against each patient's specific NTM isolate [1].

For example, for patients with *M. intracellulare* infection, treatment initially followed the standardised plan. As such, patients received oral azithromycin (300 mg/d) or clarithromycin (500 mg/d), rifampicin (450 mg/d), ethambutol (15 mg/kg-d), and injectable amikacin (400 mg, three times per week). In the precision plan, the initial dosage regimen mirrored the standard treatment, except that clarithromycin was discontinued due to DST-confirmed resistance to macrolides. Additionally, patients received a minimum

of five drugs based on DST results showing drug sensitivity, which were selected from the following list: linezolid (600 mg/d), rifampicin (450 mg/d), ethambutol (15 mg/kg-d), amikacin (400 mg, three times per week), rifabutin (300 mg/d), moxifloxacin (400 mg/d), or cefoxitin (1 g every 8 hours).

Therapeutic evaluation

Symptom assessment: Numbers of patients experiencing cough, haemoptysis, chest distress, and/or fever before treatment and after 2, 6, and 9 months of treatment were recorded and compared between the two groups.

Bacterial efficacy: Culture and smear testing was conducted on sputum specimens collected before treatment and after treatment for 2, 6, and 9 months for both groups; then results were statistically analyzed. A positive sputum smear result was defined as the detection of acid-fast bacilli in sputum, while a negative sputum smear result was indicated by three negative sputum smear results for acid-fast bacilli. A negative culture result was defined as one or more negative sputum or BALF culture results.

Imaging manifestations: Chest CT scans were taken after 6 and 9 months of treatment; and lesions on scans were counted and lesion extent was determined by calculating the proportion of lung fields containing lesions. Treatment efficacy was defined based on changes in lesion number and/or pathology, as well as on cavity changes.

Lesion number changes were categorised as follows: (i) significant absorption: absorption of over half of original lesions. (ii) absorption: absorption of less than half of the original lesion. (iii) unchanged: no significant absorption or deterioration of lesions. (iv) worsening: increase in lesion size and/or pathological changes.

Cavity changes were categorised as follows: (i) reduction: maximum cavity diameter reduced by half of the original maximum diameter. (ii) unchanged: maximum cavity diameter decreased or increased by less than half of the original maximum diameter. (iii) increase: maximum cavity diameter increased by more than or equal to half of the original maximum diameter.

Data analysis

All data were statistically analyzed using Statistical Package for the Social Sciences (SPSS) 22.0 software. The mean value was expressed as $\bar{X} \pm S$. Statistical analysis was performed using the Chi-square test and a significance level of $p < 0.05$ considered statistically significant.

Results

Strain identification results

A total of 672 NTM strains from 101 patients were successfully isolated and further cultured. As indicated in Figure 2, the most abundant NTM species was *M. intracellulare*, (493, 73.36%), followed by *Mycobacterium abscessus* (*M. abscessus*, 86, 12.80%). Only seven additional pathogenic NTM species were identified, including *Mycobacterium avium* (*M. avium*), *Mycobacterium gordonae* (*M. gordonae*), *Mycobacterium kansasii* (*M. kansasii*), *Mycobacterium columbia* (*M. columbia*), and others (Figure 2).

DST results of NTM strains

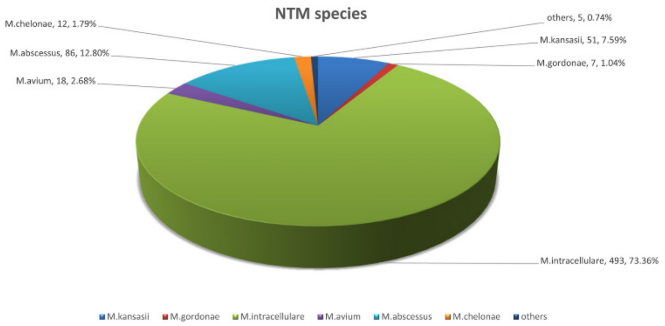
Table 1 presents antibiotic resistance rates for each NTM species present in our collection of NTMPD patient isolates. Notably, 100% of *M. avium* isolates

Table 1. Drug sensitive test results of NTM.

Antibiotics	*M. kansasii* (n = 51)		*M. gordonae* (n = 7)		*M. intracellulare* (n = 493)		*M. Columbia* (n = 1)		*M. maeseillense* (n = 3)		*M. avium* (n = 18)		*M. chelonae* (n = 12		*M. abscessus* (n = 86)		*M. fortuitum* (n = 1)	
	R	Rate	R	Rate	R	Rate	R	Rate	R	Rate	R	Rate	R	Rate	R	Rate	R	Rate
Linezolid	1	1.96	0	0.00	64	12.98	0	0.00	1	33.33	18	100.00	4	33.33	34	39.53	1	100.00
Doxycycline	50	98.04	5	71.43	479	97.16	1	100.00	2	66.67	18	100.00	12	100.00	83	96.51	1	100.00
Clarithromycin	0	0.00	0	0.00	29	5.88	0	0.00	0	0.00	1	5.56	3	25.00	18	20.93	1	100.00
Minocycline	48	94.12	3	42.86	485	98.38	1	100.00	2	66.67	18	100.00	12	100.00	77	89.53	1	100.00
Rifampicin	4	7.84	0	0.00	21	4.26	0	0.00	1	33.33	2	11.11	10	83.33	83	96.51	1	100.00
Tobramycin	46	90.20	2	28.57	53	10.75	0	0.00	0	0.00	9	50.00	10	83.33	81	94.19	1	100.00
Ethambutol	7	13.73	1	14.29	114	23.12	1	100.00	1	33.33	9	50.00	12	100.00	84	97.67	1	100.00
Gatifloxacin	13	25.49	1	14.29	254	51.52	1	100.00	3	100.00	9	50.00	10	83.33	81	94.19	0	0.00
Moxifloxacin	2	3.92	0	0.00	6	1.22	1	100.00	0	0.00	3	16.67	4	33.33	57	66.28	0	0.00
Amikacin	7	13.73	0	0.00	6	1.22	0	0.00	0	0.00	1	5.56	4	33.33	15	17.44	0	0.00
Sulfamethoxazole	51	100	5	71.43	465	94.32	1	100.00	3	100.00	18	100.00	9	75.00	82	95.35	1	100.00
Azithromycin	11	21.57	0	0.00	146	29.61	1	100.00	2	66.67	18	100.00	5	41.67	56	65.12	1	100.00
Cefoxitin	50	98.04	0	0.00	116	23.53	0	0.00	0	0.00	3	16.67	1	8.33	12	13.95	0	0.00
Rifabutin	2	3.92	0	0.00	15	3.04	0	0.00	0	0.00	1	5.56	9	75.00	60	69.77	1	100.00
Imipenem and cilastatin sodium	50	98.04	7	100	479	97.16	1	100.00	3	100.00	18	100.00	12	100.00	82	95.35	1	100.00

NTM: nontuberculous mycobacterial infection; R: resistant; Rate: drug resistance rate (%).

Figure 2. Identified NTM species percentages (n = 672).



NTM: nontuberculous mycobacterial infection.

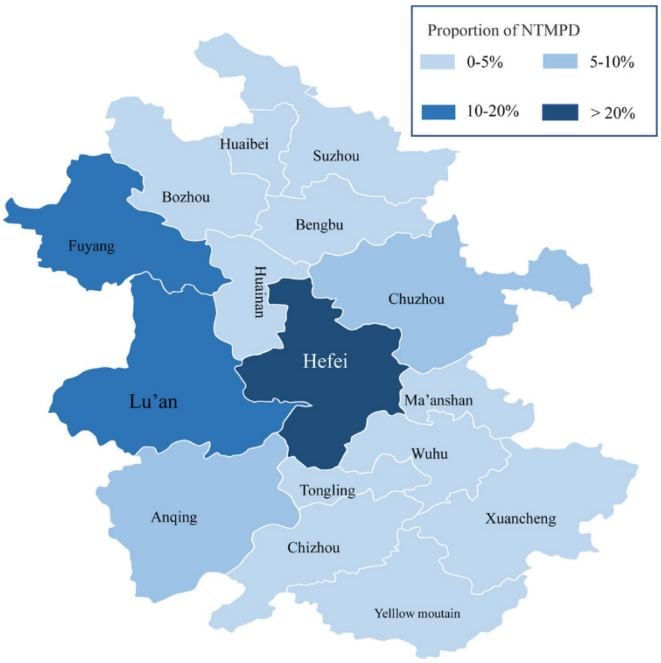
exhibited resistance to multiple drugs, while 95% of *M. abscessus* isolates exhibited resistance to most antibiotics. Additionally, 100% of NTM isolates belonged to *Mycobacterium* species *M. chelonae*, *M. columbia*, *M. marseillense*, *M. fortuitum*, and *M. gordonae*, and were resistant to imipenem-cilastatin, along with 95% of NTM isolates of other species.

An analysis of antibiotic resistance rates of *M. intracellulare* and *M. abscessus* isolates (Table 2) revealed significant differences in resistance rates to rifampin, ethambutol, moxifloxacin, and rifabutin. *M. abscessus* isolates had high-level drug resistance rates to rifampin, ethambutol, moxifloxacin, and rifabutin of 95.35%, 94.19%, 39.53%, and 69.77%, respectively. In contrast, resistance rates of *M. intracellulare* isolates to these drugs were 4.26%, 23.12%, 1.22%, and 3.04%, respectively. These rates were statistically significantly different between the two species ($p < 0.001$).

General patient clinical characteristics

Table 3 provides an overview of the general clinical characteristics of all patients. Notably, more than half of patients were affected by marasmus (57.43%), as indicated by low body mass index value. The majority of patients worked as farmers (54.46%), and the proportion of retirees ranked second. Geographically, the highest numbers of patients lived in Hefei, Lu'an, and Fuyang, accounting for 39.60%, 16.83%, and 12.87% of patients, respectively (Figure 3). Approximately 26.73% of patients were illiterate.

Figure 3. Geographic distribution of NTMPD prevalence in Anhui Province.



NTMPD: nontuberculous mycobacterial pulmonary disease.

Table 2. Drug sensitive test results of *M. intracellulare* and *M. abscessus*.

Antibiotics	*M. intracellulare* (n = 493)		*M. abscessus* (n = 86)		χ^2	*p* value
	N	Rate	N	Rate		
Linezolid	64	12.98	11	12.79	0.002	0.961
Doxycycline	479	97.16	79	91.86	5.884	0.015
Clarithromycin	29	5.88	12	13.95	7.25	0.007
Minocycline	485	98.38	74	86.05	33.386	<0.001
Rifampicin	21	4.26	82	95.35	415.445	<0.001
Tobramycin	53	10.75	57	66.28	146.721	<0.001
Ethambutol	114	23.12	81	94.19	165.553	<0.001
Gatifloxacin	254	51.52	70	81.40	26.517	<0.001
Moxifloxacin	6	1.22	34	39.53	167.177	<0.001
Amikacin	6	1.22	3	3.49	2.469	0.116
Sulfamethoxazole	465	94.32	76	88.37	4.225	0.041
Azithromycin	146	29.61	34	39.53	3.364	0.067
Cefoxitin	116	23.53	9	10.47	7.383	0.007
Rifabutin	15	3.04	60	69.77	289.139	<0.001
Imipenem and cilastatin	479	97.16	82	95.35	0.798	0.372

N: number of resistant strains; Rate: drug resistance rate (%).

Regarding symptoms, cough was present in 89.11% of patients, and 42.57% reported breathing distress, while a significant proportion experienced haemoptysis and fever. Importantly, 47.52% had histories of TB infection, 77.23% had hypoproteinemia, 69.31% had anaemia, and 38.61% had history of surgeries. Only 6% had comorbidities such as hypertension, diabetes, and heart disease. Laboratory tests revealed anti-MTB antibodies in sera of 22 patients and positive blood Tuberculosis-Spot (TSPOT) results for four patients. Additionally, imaging findings indicated signs of pulmonary bronchiectasis in 66 patients and cavities in 54 patients.

Table 3. Clinical data analysis of NTMPD in Anhui Province.

Characteristics	n	%
Gender		
Female	48	47.52
Male	53	52.48
Age (years)		
≥ 60	58	57.43
< 60	43	42.57
BMI		
< 18.5	58	57.43
18.5-23.9	41	40.59
≥ 24	2	1.98
Occupation		
Farmer	55	54.46
Retiree	18	17.82
Worker	8	7.92
Clerk	4	3.96
Education*	3	2.97
Other	13	12.87
Illiteracy		
Yes	27	26.73
No	74	73.27
Clinical symptoms		
Cough	90	89.11
Chest distress	43	42.57
Haemoptysis	32	31.68
Fever	23	22.77
Night sweat	14	13.86
Asymptomatic**	14	13.86
Thoracalgia	13	12.87
Complications		
Hypertension	6	5.94
Diabetes	5	4.95
Cardiopathy	4	3.96
Tuberculosis history	48	47.52
Surgery history	39	38.61
Tumor history	11	10.89
COPD	10	9.90
Hypoproteinemia	78	77.23
Anemia	70	69.31
Electrolyte disturbance	49	48.51
Immunological index		
Serum tuberculosis antibody	22	21.78
TSPOT	4	3.96
Imaging features		
Bronchiectasis	66	65.35
Cavity	54	53.47

* students and teachers; ** only found in physical examination. BMI: body mass index; COPD: chronic obstructive pulmonary disease; TSPOT: Tuberculosis-SPOT test.

Therapeutic observation

Among the 101 NTMPD patients included in this study, 73 were infected with *M. intracellulare* and 28 with *M. abscessus*. Patient symptoms, sputum culture results, and imaging findings are summarised in Tables 4-6. Overall, no significant differences in rates of fever, cough, and haemoptysis symptoms were observed between groups receiving standardised and precision treatments after the initial standardised treatment period. However, after 9 months of treatment, the precision treatment group exhibited a higher symptoms remission rate as compared to that of the standardised treatment group.

Our results also showed a marked decrease in the proportion of patients with positive sputum smear results in both treatment groups after 6 months of antibiotic therapy as compared to baseline results (obtained before treatment initiation). Nevertheless, no statistically significant intergroup differences were observed after 2, 6, or 9 months of treatment. Similarly, although the proportion of positive sputum culture results also decreased with increasing treatment duration, no statistically significant intergroup differences were observed after 9 months of treatment.

Analysis of imaging findings revealed no significant differences in lesion severity or cavity changes between the two groups after 6 months of treatment, although lesions of one patient in the standardised treatment group and two patients in the precision treatment group worsened. After 9 months of treatment, the standardised treatment group exhibited a statistically significant higher lesion absorption rate as compared to that of the precision treatment group. However, no significant difference in cavity changes was observed between the two groups (Figure 4).

During the data collection process, 10 patients were excluded from the treatment efficacy analysis due to serious adverse reactions (Supplementary Table 1). Adverse drug-induced reactions were detected in a small proportion of patients; generally mild, and mainly included drug-induced liver damage. Adverse reactions improved after symptomatic treatment (Supplementary Table 2).

Discussion

The results of this retrospective cohort study provide information regarding the prevalence and clinical characteristics of NTM infections occurring in a representative population of Anhui Province.

Table 4. Clinical symptoms.

Treatment	Before treatment				2 months				6 months				9 months			
	Cough	Chest distress	Haemoptysis	Fever	Cough	Chest distress	Haemoptysis	Fever	Cough	Chest distress	Haemoptysis	Fever	Cough	Chest distress	Haemoptysis	Fever
Standardized plan (n = 53)	46 (86.79)	14 (26.42)	23 (43.40)	11 (20.75)	43(81.13)	4 (7.55)	12 (22.64)	5 (9.43)	44 (83.02)	3 (5.66)	5 (9.43)	1 (1.89)	41 (77.36)	4 (7.55)	3 (5.66)	3 (5.66)
Precision plan (n = 48)	44 (91.67)	18 (37.50)	20 (41.67)	12 (25.00)	42 (87.50)	11 (22.92)	19 (39.58)	8 (16.67)	37 (77.08)	8 (16.67)	12 (25.00)	2 (4.17)	33 (68.75)	2 (4.17)	10 (20.83)	0 (0.00)
χ^2	1.998				3.821				5.789				7.957			
p value	0.573				0.281				0.122				0.047			

n (%): number (percentage).

Table 5. Bacteriological efficacy.

Specimen type	Treatment	Before treatment	2 months	6 months	9 months
Positive sputum smear	Standardized plan (n = 53)	26 (49.06)	15 (28.30)	17 (32.08)	12 (22.64)
	Precision plan (n = 48)	27 (56.25)	18 (37.50)	7 (14.58)	13 (27.08)
	χ^2	0.523	0.969	4.254	0.267
	p value	0.470	0.325	0.039	0.605
Positive sputum culture	Standardized plan (n = 53)	49 (92.45)	25 (47.17)	22 (41.51)	24 (45.28)
	Precision plan (n = 48)	42 (87.50)	26 (54.17)	13 (27.08)	16 (33.33)
	χ^2	0.693	0.493	2.315	1.504
	p value	0.405	0.482	0.128	0.220

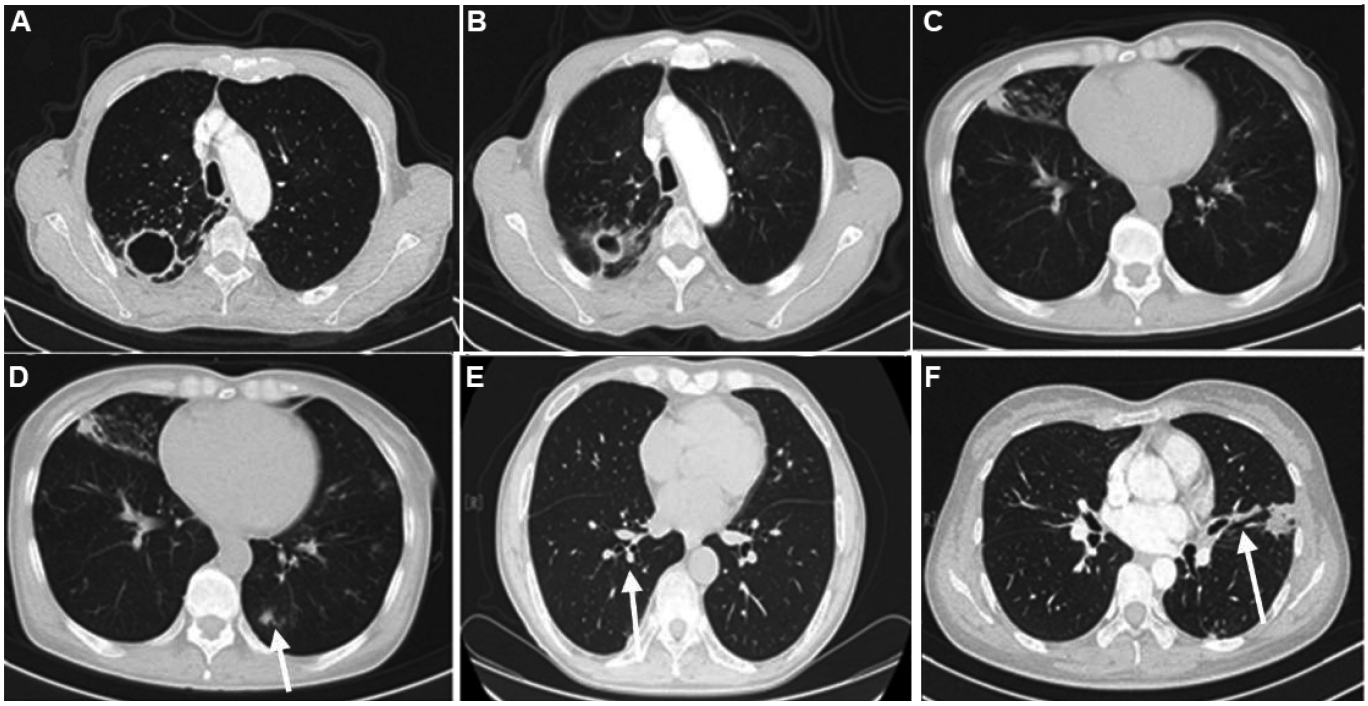
n (%): number (percentage).

Table 6. Imaging changes after treatment.

	6 months									9 months								
	Lesions change					Cavity change				Lesions change					Cavity change			
Treatment	SA	A	UC	W	IR (%)	R	UC	I	IR (%)	SA	A	UC	W	IR (%)	R	UC	I	IR (%)
Standardized plan (n = 53)	2	8	42	1	18.87	2	21	0	9.44	7	20	25	1	50.94	4	19	0	19.18
Percentage (%)	3.77	15.09	79.25	1.89		8.70	91.0	0.00		13.21	37.74	47.17	1.89		17.39	82.61	0.00	
Precision plan (n = 48)	3	6	37	2	18.75	3	28	0	10.22	9	7	32	0	33.33	1	30	0	4.26
Percentage (%)	6.25	12.50	77.08	4.17		9.68	90.32	0.00		18.75	14.58	66.67	0.00		3.23	96.77	0.00	
χ^2			0.890				0.015					8.141				3.153		
p value			0.828				0.902					0.043				0.076		

SA: significant absorption; A: absorption; UC: unchanged; W: worse: IR: improvement rate; R: reduction; I: increase; n (%): number (percentage).

Figure 4. Partial CT images of NTMPD patients.



A. Cavity in NTMPD patients. **B.** Reduction of cavity after 9 months' precise treatment. **C, D.** The lung lesions were aggravated after 6 months' standardized treatment. **E, F.** Bronchiectasis in different patients. CT: computed tomography; NTMPD: nontuberculous mycobacterial pulmonary disease.

In particular, we also compared the therapeutic effectiveness of two NTMPD patient treatment plans as guided by DST results in the real-world setting of our hospital. Our results demonstrated that treatment of NTM infections can be challenging, thus suggesting that selection of an appropriate empiric therapeutic schedule is of equal importance to the use of DST results to guide the formulation of a suitable antimicrobial treatment regimen.

It is well known that the distribution of NTMs exhibits significant regional diversity. For example, in Europe and North America, NTM-infected patients are frequently found to be infected with organisms belonging to the *Mycobacterium avium* Complex (MAC), *M. gordonae*, and *M. bufo* species. Meanwhile, in South America, predominant opportunistic *Mycobacterium* species isolated from NTM-infected patients include MAC, *M. kansasii*, and *M. gordonae*, while in Asia isolated NTM species are predominantly MAC, *M. abscessus*, and other opportunistic *Mycobacterium* species [13].

Notably, results of the current study suggest that the dominant NTM species in Anhui Province are primarily *M. intracellulare* and *M. abscessus*, as consistent with findings obtained in Nanjing by Hu *et al*. [14]. However, these results significantly differ from those obtained from NTM patients in Chongqing, Shanghai, and other regions of China, which collectively suggest that notable regional NTM distribution differences exist across China [15,16].

The results of this study also highlight the complexity of NTM drug resistance profiles in Anhui Province and raises serious concerns. Importantly, all NTM isolates analyzed in this study exhibited extreme high-level resistance to imipenem-cilastatin, a commonly used antibiotic in clinical settings. The mechanism of resistance has not been fully elucidated.

Accumulated evidence indicates that NTM exhibit inherent antibiotic resistance through various mechanisms. This resistance may arise from effects of robust NTM cell walls, and/or surrounding biofilm and granuloma-associated barriers with limited drug permeability that reduce NTM drug exposure and drug uptake. Additionally, NTM may possess capabilities supporting enhanced drug efflux, heightened drug metabolism, and/or decreased drug sequestration, and may express proteins that selectively target common antibiotics. Moreover, mutations of the *rpoB* gene, a target of rifampicin, are the main cause of acquired drug resistance in both *M. avium* complex and *M. kansasii*. Furthermore, rifampicin has been shown to preferentially inhibit transcription of one of the two *rpoB* promoters, leading to increased transcription of the other promoter that supports the development of strains with enhanced rifampicin resistance [17]. Consequently, these drugs should be administered with caution to patients with suspected or confirmed NTM infections.

An analysis of patient clinical characteristics revealed that NTMPD patients in Anhui were predominantly elderly male farmers, which aligns with the results of another study conducted in South Korea [18]. These observations may be partly explained by the fact that men tend to have higher numbers of daily contacts with soil than women [19]. Furthermore, more than half of NTMPD patients exhibited marasmus, as consistent with observations reported by Blakney *et al.* [20], while a large proportion of our patients suffered from hypoproteinemia and anaemia. Taken together, these findings suggest a possible relationship between nutritional status and development of NTMPD [21].

While the relationship between NTM drug sensitivity and treatment effects has not been extensively discussed previously [22], the results of this study indicate that DST-guided customisation of treatment did not provide obvious advantages. Nevertheless, recommended best practices dictate that once patients are diagnosed with NTMPD, they should receive standardised therapies according to recommended guidelines as soon as possible in order to avoid increased economic and psychological burdens associated with delayed treatment.

Limitations

There were several limitations in this study. This project was a single-centre retrospective study that generated results that may not be applicable to populations outside of Anhui Province. Additionally, the treatment follow-up period of both of our treatment groups was at most 9 months, shorter than the duration of the standard NTM treatment regimen. Moreover, no assessments of treatment-induced organ damage were conducted for use in evaluating and comparing the safety of NTM treatments. Finally, our analysis did not include additional factors, such as drug combination, treatment durations of the different medications, or patient treatment compliance as potentially influential factors on patient treatment outcomes. Additional prospective, controlled studies are needed to evaluate the effects of these factors on patient prognosis and validate the findings of the current study.

Conclusions

The results of this study demonstrated that *M. intracellulare* and *M. abscessus* may be dominant opportunistic pathogenic NTM species in Anhui Province, China. Furthermore, our results revealed the rates of NTM resistance to multiple drugs, including rifampicin, amikacin, and others. Additionally, most of our NTMPD patients were elderly, marasmic males working as farmers. Most importantly, our results emphasise that treatment of patients with NTM infections can be challenging, such that selection of an appropriate empiric therapeutic schedule is of equal importance as the use of DST to customise the formulation of an appropriate antimicrobial treatment regimen.

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Data availability

We confirm that the manuscript has not been published elsewhere and is not under consideration by other journals. The data used to support the findings of this study are available from the corresponding author upon request.

Authors' contributions

WHL, conceptualized and designed the study; ZYZ and SSL, organized the data and performed data analysis; ZYZ and LG, wrote the first draft of the manuscript; YYL, wrote sections of the manuscript; DPW and DFX, conducted laboratory tests; YL and JBL, provided technical assistance. All authors have contributed significantly to the work, and have read and agreed on the content of the final version of the manuscript. ZYZ and LG contributed equally to this article.

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Annex – Supplementry Items

Supplementary Table 1. Cases with serious adverse reaction.

Reaction	Number of cases
Drug-induced liver injury	6
Thrombocytopenia	2
Emesis	1
Hearing impairment	1

Supplementary Table 2. Cases with adverse reactions in the different treatment groups.

Reaction	Standardised treatment (n = 53)	Precision treatment (n = 48)
Drug-induced liver injury	38	35
Emesis	19	28
Drug fever	18	20
Leukopenia	17	26
Diarrhea	7	16
Thrombocytopenia	5	8
Hearing impairment	1	4
Drug eruption	3	1
Visual impairment	1	2