JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Distribution of non-tuberculous *Mycobacteria* strains and analysis of drug resistance in Lishui City, China

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Abstract

Introduction: This study aimed to investigate the distribution and drug resistance of clinically isolated *Non-tuberculous Mycobacteria* (NTM) strains in Lishui City, and to provide a basis for the development of NTM disease prevention and control strategies in the region.
Methodology: This retrospective cross-sectional study was performed using NTM-positive clinical specimens collected from patients at a sentinel tuberculosis hospital in Lishui City, Zhejiang Province, China, between January 2023 and December 2024. The isolated NTM strains were identified using a gene chip–based method. Antimicrobial susceptibility testing was conducted using the microbroth dilution method. Statistical analyses were applied to evaluate the isolation rate, species distribution, and drug-resistance patterns of the strains.
Results: The isolation rate of NTM among clinical patients at the sentinel hospital was 18.09% (157/868); The rate was significantly higher in women (26.97%, 72/267) than in men (14.14%, 85/601) (χ^2 trend = 20.518, $p < 0.001$); Nine common NTM species were identified, with *Mycobacterium intracellulare* being the most prevalent (68.79%, 108/157), followed by *Mycobacterium avium* (11.46%, 18/157) and *Mycobacterium chelonae/abscessus* (8.28%, 13/157). Antimicrobial susceptibility testing revealed high resistance rates to several drugs, including imipenem (94.27%, 148/157), doxycycline (94.27%, 148/157), rifampicin (91.72%, 144/157), amoxicillin/clavulanic acid (91.08%, 143/157), meropenem (89.81%, 141/157), cefoxitin (87.90%, 138/157), minocycline (85.99%, 135/157), and ciprofloxacin (85.99%, 135/157). Species-specific differences in resistance patterns were observed.
Conclusions: The distribution of clinically isolated NTM strains in Lishui City was dominated by *Mycobacterium intracellular*, *Mycobacterium avium,* and *Mycobacterium chelonae/abscessus*. These isolates demonstrated high levels of resistance to commonly used antimicrobial agents, highlighting the need for improved treatment and control strategies.

Key words: non-tuberculous mycobacteria; gene chip; species identification; antimicrobial resistance.

J Infect Dev Ctries 2026; 20(3):435-444. doi:10.3855/jidc.21596

(Received 13 March 2025 – Accepted 24 June 2025)

Introduction

Non-tuberculous mycobacteria (NTM) are opportunistic pathogens that include more than 190 species, excluding the *Mycobacterium tuberculosis complex* and *Mycobacterium leprae*. Only a subset of these species is considered clinically relevant [1-5]. With advances in tuberculosis control and molecular diagnostics, the public health importance of NTM infection has increased, and NTM is now recognized as a causative agent of pulmonary, cutaneous, and mucosal diseases [6-9]. Epidemiological studies have reported a steady global increase in both the incidence and mortality of NTM disease [10]. In China, the isolation rate of NTM has also demonstrated a marked upward trend [11-13], rising from 4.24% to 12.68% between 2014 and 2021 [14].

Given the substantial geographical differences in NTM species distribution and drug-resistance profiles [1,15,16], a systematic understanding of region-specific epidemiological characteristics is essential for optimizing clinical diagnosis and treatment protocols, as well as for formulating effective prevention and control strategies. Currently, there is a paucity of research on NTM infection and drug resistance in the population of southwest Zhejiang, Lishui City, as a typical mountainous prefecture-level city, which possesses a unique geographical environment and an aging population structure that may contribute to distinct epidemiological features of NTM. Therefore, in this study, NTM strains isolated from clinical patients in Lishui between January 2023 and December 2024 were identified using a gene chip–based method, and

antimicrobial susceptibility testing of clinically relevant drugs was performed using the microbroth. By analyzing the distribution of dominant species and their drug-resistance characteristics, this study aims to establish a region-specific NTM pathogen spectrum and drug resistance database, which will provide a scientific basis for medical institutions to develop individualized treatment plans and public health departments to improve the prevention and control system.

It should be acknowledged that the specimens analyzed in this study were exclusively obtained from respiratory sources, including sputum and bronchoalveolar lavage fluid. As a result, the findings may not fully reflect the spectrum of NTM infections occurring at extrapulmonary sites. Therefore, conclusions regarding the overall epidemiological status and drug-resistance patterns of NTM in Lishui should be interpreted with caution.

Methodology

Samples and strains

Respiratory samples, including sputum and fiberoptic bronchoscopic alveolar lavage fluid, were collected from patients with suspected mycobacterial infection who attended a tuberculosis sentinel medical institution in Lishui City, Zhejiang Province, from January 2023 to December 2024. The samples were initially screened and cultured by the BACTEC MGIT 960 fully automated mycobacterial culture system, and the positive strains were initially typed and identified using the *Mycobacterium tuberculosis* Complex Group Antigen Colloidal Gold Immunochromatography Kit after the positive strains were confirmed to be acid-resistant bacilli by Celcium-Ni antacid staining review. After exclusion of duplicate isolates from the same patient, the strains were included for further drug sensitivity testing for subsequent analytical studies.

Inclusion criteria: 1) The finally included cases are those with positive culture results for ≥ 2 times and confirmed by strain identification; 2) Patients diagnosed with NTM disease, according to the 2020 Chinese diagnostic guidelines, were included in this study. Patients with HIV infection and organ transplantation are excluded.

Exclusion criteria: Duplicate strains from the same patient, specimens with negative cultures or contamination, or strains identified as *Mycobacterium tuberculosis* complex.

Ethics Statement

This study was a retrospective analysis based on anonymized laboratory data obtained from routine clinical diagnostics. Ethical approval was obtained from the Ethics Committee of Lishui City Tuberculosis Sentinel Hospital. Approval No.: Clinical Ethics Review (KY-2024053).

Instruments and reagents

MGIT 960 *Mycobacterium* species rapid culture instrument (Model: MGIT 960, BD, USA); Bacterial ultrasonic dispersion counter (Model: 1100c, Guangdong Sig Biotechnology Co., Ltd.); Brio LifeTouch Gene Amplifier (Model: TC - 96/G/H (b) B, Hangzhou Brio Technology Co. BioMixer Microarray Hybridization Instrument (Model: BioMixerTMII, Beijing Boao Biotechnology Co., Ltd.); VIVOTEK LuxScan10K-B Microarray Microarray Scanner (Model: LuxScan-10K/B, Beijing Boao Biotechnology Co., Ltd.); Microbial Sampling Autosampler (Model: BSJ-9612, Zhuhai Besso BioTech Co. Ltd.); Microbial drug sensitivity analysis system (Model: BSP - TB96, Zhuhai Beso Biotechnology Co.)

MGIT 960 *Mycobacterium* culture kit (Item No. CNL20B0032, BD Company, USA), antacid staining reagent (Item No. BA4091, Zhuhai Beso Biotechnology Co., Ltd.); *Mycobacterium tuberculosis* antigen detection kit (Item No. P111006, Hangzhou Innovative Bio-Technology Co., Ltd.); identification reagent of *Mycobacterium* species based on the DNA microarray microarray (Lot No. 20). Identification reagent (Batch No.: 20220401, Chengdu Boao Jingxin Biotechnology Co., Ltd.); Non-tuberculosis *Mycobacterium tuberculosis* drug sensitivity detection kit (Item No.: BC8101, Zhuhai Beso Biotechnology Co., Ltd.).

Culture and preliminary identification of mycobacteria

Mucus sputum, concentrated sputum specimens, and centrifugation-treated alveolar lavage were taken and treated with freshly prepared digestive solution (containing 3% NaOH, 1.45% sodium citrate, 0.5% cysteine) for 15 minutes. The supernatant was discarded after centrifugation at 3000 × g for 20 min, and the precipitate was resuspended in phosphate buffer. 500 μL of the suspension was inoculated into special culture tubes containing OADC nutrient additives and placed in the *Mycobacterium* automated culture system for incubation. Culture-positive specimens were firstly prepared as colony smears for microscopic examination with Celcite-Nicotinic acid staining, and then *Mycobacterium tuberculosis*-specific antigen detection reagents were used for complex group identification, while antigen detection-negative strains were identified by gene chip technology, and the first detection was included in the statistics of the repeated testing data for

the same patient if the same NTM strains were detected several times.

Species identification (gene chip method)

Add the nucleic acid extraction solution into a 1.5 mL centrifuge tube and collect samples of non-tuberculosis mycobacterial strains from the selected culture medium, and then complete the nucleic acid extraction through shaking, water bath, centrifugation, and other operations. The PCR reaction system was prepared by mixing 18 μL of PCR amplification reagent with 2 μL of template DNA, and then amplified in the BORI amplifier. After amplification, the PCR product was placed in the PCR instrument and denatured at 95°C for 5 min, followed by cooling in an ice water bath for 3 min. Pipette 6 μL of PCR amplification product and add it to an eight-row tube containing 9 μL/tube of hybridization buffer. According to the procedure of the identification kit, 14 μL of hybridization mixture was aspirated and added to the microarray through the spiking well. The chip was hybridized at 50°C for 2 hours at 5 r/min. After hybridization, the chip is rinsed with the matching washing solution, dried, and put into the chip scanner to determine the results of strain identification. The test covers 17 common clinical mycobacterial species or groups, including *Mycobacterium tuberculosis complex*, *Mycobacterium tuberculosis complex, Mycobacterium intracellulare, Mycobacterium avium, Mycobacterium gordonae, Mycobacterium kansasii, Mycobacterium fortuitum, Mycobacterium scrofulaceum, Mycobacterium gilvum, Mycobacterium terrae, Mycobacterium chelonae and Mycobacterium abscessus, Mycobacterium phlei, Mycobacterium nonchromogenicum, Mycobacterium marinum and Mycobacterium ulcerans, Mycobacterium aurum, Mycobacterium szulgai and Mycobacterium malmoense, Mycobacterium xenopi, Mycobacterium smegmatis*).

Drug susceptibility testing of non-tuberculous mycobacteria

The selected NTM strains were subjected to drug sensitivity tests for 16 drugs by the liquid-based microtiter plate method. Ultrasonic turbidimetric quantification of bacteria was carried out by applying a bacterial ultrasonic dispersion counter, and the operation was interpreted according to the instructions of the drug sensitivity test kit (microtiter plate method). NTM drug sensitivity plates were prepared, containing different drugs and the minimum inhibitory concentration (MIC) resistance folds were: amikacin ≥ 64 μg/mL; amoxicillin/clavulanic acid ≥ 32/16 μg/mL; cefoxitin ≥ 128 μg/mL; minocycline ≥ 8 μg/mL; doxycycline ≥ 8 μg/mL; ciprofloxacin ≥ 4 μg/mL; clarithromycin (slow-growth NTM ≥ 32 μg/mL, fast-growth NTM ≥ 8 μg/mL); rifampicin ≥ 2 μg/mL; rifabutin ≥ 4 μg/mL; imipenem ≥ 32 μg/mL; linezolid ≥ 32 μg/mL; moxifloxacin ≥ 4 μg/mL; teicoplanin > 4 μg/mL; tobramycin ≥ 8 μg/mL; trimethoprim/sulfamethoxazole ≥ 4/76 μg/mL; Meropenem ≥ 32μg/mL; inoculation solution and other reagents and materials, first prepare and dilute the bacterial suspension (can be obtained from liquid or solid medium, adjusted to 0.5MCF standard turbidity), then dilute the bacterial suspension and inoculation (take out the drug sensitivity plate and inoculation solution to return to room temperature in advance, mix the bacterial suspension, then add the OADC according to the requirements of the inoculation), and then incubation (fast-growing mycobacteria 30 ℃ ± 2 ℃) Then culture (fast-growing mycobacteria 30℃ ± 2℃ for 72 hours or longer, slow-growing mycobacteria 35℃ ± 2℃ for 7 days or 14 days, pay attention to the conditions and the number of stacked layers). At the end of the incubation, use the microbial drug sensitivity analysis system according to the product instructions to automatically read the MIC value, and automatically read the drug sensitivity results according to the system's built-in folding point program.

Statistical analysis

Excel 2021 was used to establish the database, and SPSS 27.0 software was used to statistically analyze the data, and the count data were described by "percentage or constitutive ratio (%)"; comparisons of differences between groups were tested by the chi-square test or Fisher's exact probability method, and the trends of the detection rate and the resistance rate over time were tested by the trend chi-square test. Differences were considered statistically significant at $p < 0.05$.

Results

Isolation, culture, and identification of mycobacteria

A total of 868 strains of *Mycobacterium* spp. were isolated and cultured from 2023 to 2024, among which the strains that were antigen-negative for *Mycobacterium tuberculosis* were identified as NTM strains, and 157 strains were identified as NTM strains, with an NTM isolation rate of 18.09% (157/868); among them, 85 strains were detected in males, and 72 strains were detected in females; the NTM detection rate in males was 14.14% (85/601), lower than the female NTM detection rate of 26.97% (72/267), and the

Table 1. Comparison of NTM Detection Rates by Demographic Characteristics.

Basic characteristics	Group	Number of mycobacteria (strains)	Number of Mycobacterium tuberculosis (strains)	Number of non-tuberculous mycobacteria (strains)	Proportion of non-tuberculous mycobacteria (%)	χ^2	p
Gender	Male	601	516	85	14.14	20.518	< 0.001
	Female	267	195	72	26.97		
Age (years)	Non-Elderly Group (< 60)	377	331	46	12.20	15.584	< 0.001
	Elderly Group (≥ 60)	491	380	111	22.61		
Year	2023	446	366	80	17.94	0.014	0.906
	2024	422	345	77	18.25		
Total		868	711	157	18.09		

The original age groups were 1–19, 20–39, 40–59, and ≥60 years. However, since the total number of NTM cases in the 1–19 and 20–39 age groups was only two, the grouping was optimized into two categories: Elderly Group (≥60 years) and Non-Elderly Group (<60 years).

difference was statistically significant ($\chi^2 = 20.518$, $p < 0.001$); Age was categorized into four groups (1–19, 20–39, 40–59, and ≥ 60 years) based on standard epidemiological age bands commonly used in respiratory infection studies and to reflect differences in NTM susceptibility by age-related immune status and comorbidity prevalence; among all age groups, the elderly group aged 60 years and above had the highest NTM detection rate of 22.61% (111/491), and the difference in NTM detection rate among age groups was statistically significant ($\chi^2 = 31.188$, $p < 0.001$) (Table 1).

Species distribution of nontuberculous mycobacteria

Among 157 NTM isolates, nine known species were identified, predominantly *M. intracellulare*, *M. avium*, and *M. chelonae/abscessus*, with *Mycobacterium intracellulare* being the most common with a 68.79% (108/157), followed by *Mycobacterium avium* with an 11.46% (18/157) and *Mycobacterium chelonae/abscessus* with an 8.28% (13/ 157), and a few isolates of *Mycobacterium szulgai/Malmoense*, *Mycobacterium kansasensis*, etc., the three major NTM species (including *Mycobacterium avium/intracellulare complex*) accounted for more than 90% of the total number of NTM strains, and the detailed composition ratios of non-tuberculous mycobacteria in different years, are shown in Table 2.

Drug resistance in non-tuberculous mycobacteria

Sixteen antibiotic drug sensitivity tests were performed on 157 NTM strains using the micro broth dilution method, and the results showed that the drugs with higher overall NTM resistance rates were: imipenem 94.27% (148/157), doxycycline 91.72% (144/157), rifampicin 91.72% (144/157), amoxicillin/clavulanic acid 91.08% (143/157), and meropenem 89.81% (141/157); among them, the drugs with higher rates of slow-growth NTM resistance were imipenem 95.10% (136/143), doxycycline 91.61% (131/143), rifampicin 90.91% (130/143), amoxicillin/clavulanic acid 90.21% (129/ 143), and cefoxitin 90.21% (129/143); drugs with lower resistance rates were rifabutin 1.40% (2/143), clarithromycin 2.80% (4/143), amikacin 3.50% (5/143),

Table 2. Distribution of NTM strains in Lishui City, 2023-2024.

Antimicrobials	Mycobacterium intracellulare (n = 108)		Mycobacterium avium (n = 18)		Mycobacterium avium/intracellulare (n = 8)		Other Mycobacteria (n = 9)		Total (n = 143)	
	Drug resistance	Rate (%)	Drug resistance	Rate (%)	Drug resistance	Rate (%)	Drug resistance	Rate (%)	Drug resistance	Rate (%)
Amikacin	5	4.63	0	0.00	0	0.00	0	0.00	5	3.50
Amoxicillin/clavulanic acid	105	97.22	11	61.11	5	62.50	8	88.89	129	90.21
Rifampicin	105	97.22	15	83.33	4	50.00	6	66.67	130	90.91
Cefoxitin	106	98.15	12	66.67	5	62.50	6	66.67	129	90.21
Rifabutin	2	1.85	0	0.00	0	0.00	0	0.00	2	1.40
Ciprofloxacin	98	90.74	13	72.22	8	100	5	55.56	124	86.71
Clarithromycin	4	3.70	0	0.00	0	0.00	0	0.00	4	2.80
Doxycycline	103	95.37	13	72.22	7	87.50	8	88.89	131	91.61
Imipenem	106	98.15	15	83.33	6	75.00	9	100	136	95.10
Linezolid	62	57.41	7	38.89	2	25.00	2	22.22	73	51.05
Minocycline	102	94.44	11	61.11	3	37.50	6	66.67	122	85.31
Moxifloxacin	26	24.07	4	22.22	1	12.50	1	11.11	32	22.38
Tigecycline	104	96.30	9	50.00	3	37.50	6	66.67	122	85.31
Tobramycin	30	27.78	3	16.67	0	0.00	4	44.44	37	25.87
Trimethoprim/sulfamethoxazole	9	8.33	3	16.67	0	0.00	4	44.44	16	11.19
Meropenem	105	97.22	11	61.11	5	62.50	7	77.78	128	89.51

Table 3. Resistance of Slow-growing NTM to 16 different antibiotics.

Bacterial species	2023		2024		Total	
	Number of strains	Constituent ratio (%)	Number of strains	Constituent ratio (%)	Number of strains	Constituent ratio (%)
Mycobacterium intracellulare	56	70.00	52	67.53	108	68.79
Mycobacterium avium	4	5.00	14	18.18	18	11.46
Mycobacterium chelonae/abscessus	8	10.00	5	6.49	13	8.28
Mycobacterium avium/intracellulare	7	8.75	1	1.30	8	5.10
Mycobacterium spp. (species not detected)	3	3.75	0	0.00	3	1.91
Mycobacterium kansasii	1	1.25	1	1.30	2	1.27
Mycobacterium szulgai/Malmoense	0	0.00	2	2.60	2	1.27
Mycobacterium terrae	1	1.25	1	1.30	2	1.27
Mycobacterium fortuitum	0	0.00	1	1.30	1	0.64
Total	80	100.00	77	100.00	157	100.00

trimethoprim/sulfamethoxazole 11.19% (16/143), and moxifloxacin 22.38% (32/143) (Table 3).

Drugs with higher fast-growing NTM resistance rates were amoxicillin/clavulanic acid 100.00% (14/14), rifampicin 100.00% (14/14), doxycycline 92.86% (13/14), minocycline 92.86% (13/14), moxifloxacin 92.86% (13/14) and meropenem 92.86% (13/14); drugs with lower resistance rates were amikacin 0.00% (0/14), tigecycline 21.43% (3/14) and clarithromycin 28.57% (4/14) (Table 4).

To further explore the differences in antimicrobial susceptibility between slow-growing and fast-growing NTM isolates, we performed subgroup analyses and statistical comparisons. Fast-growing NTM exhibited significantly higher resistance rates to multiple antibiotics compared to slow-growing NTM. Notably, fast-growing species demonstrated markedly increased resistance to rifabutin (85.71% vs. 1.40%, $p = 0.000$), clarithromycin (28.57% vs. 2.80%, $p = 0.002$), moxifloxacin (92.86% vs. 22.38%, $p = 0.000$), tigecycline (21.43% vs. 85.31%, $p = 0.000$), tobramycin (78.57% vs. 25.87%, $p = 0.000$), and trimethoprim/sulfamethoxazole (85.71% vs. 11.19%, $p = 0.000$). Additionally, significant differences were observed for cefoxitin (64.29% vs. 90.21%, $p = 0.015$). These results highlight the considerable variation in drug resistance profiles between the two major NTM growth types (Table 5).

In addition, species-level comparisons were conducted between the two most prevalent slow-growing NTM: *Mycobacterium intracellulare* (n = 108) and *Mycobacterium avium* (n = 18). Statistically significant differences in resistance rates were found for amoxicillin/clavulanic acid (97.22% vs. 61.11%, $p = 0.000$), rifampicin (97.22% vs. 83.33%, $p = 0.038$), cefoxitin (98.15% vs. 66.67%, $p = 0.000$), ciprofloxacin (90.74% vs. 72.22%, $p = 0.041$), doxycycline (95.37% vs. 72.22%, $p = 0.006$), imipenem (98.15% vs. 83.33%, $p = 0.021$), minocycline (94.44% vs. 61.11%, $p = 0.000$), tigecycline (96.30% vs. 50.00%, $p = 0.000$), and meropenem (97.22% vs. 61.11%, $p = 0.000$). These findings indicate significant interspecies heterogeneity in antimicrobial susceptibility even among slow-growing NTM (Table 6).

Comparison of Antimicrobial Resistance by Gender and Age Group

To investigate potential sex-related differences in NTM drug resistance, we conducted subgroup analyses based on patient gender.

Table 4. Resistance of Fast-growing NTM to 16 different antibiotics.

Antimicrobials	Mycobacterium chelonae / abscessus (n = 13)		Mycobacterium fortuitum (n = 1)		Total (n = 14)	
	Drug resistance	Rate (%)	Drug resistance	Rate (%)	Drug resistance	Rate (%)
Amikacin	0	0.00	0	0.00	0	0.00
Amoxicillin/clavulanic acid	13	100.00	1	100.00	14	100.00
Rifampicin	13	100.00	1	100.00	14	100.00
Cefoxitin	9	69.23	0	0.00	9	64.29
Rifabutin	12	92.31	0	0.00	12	85.71
Ciprofloxacin	11	84.62	0	0.00	11	78.57
Clarithromycin	4	30.77	0	0.00	4	28.57
Doxycycline	13	100.00	0	0.00	13	92.86
Imipenem	12	92.31	0	0.00	12	85.71
Linezolid	11	84.62	0	0.00	11	78.57
Minocycline	13	100.00	0	0.00	13	92.86
Moxifloxacin	13	100.00	0	0.00	13	92.86
Tigecycline	3	23.08	0	0.00	3	21.43
Tobramycin	11	84.62	0	0.00	11	78.57
Trimethoprim/sulfamethoxazole	11	84.62	1	0.00	12	85.71
Meropenem	13	100.00	0	0.00	13	92.86

Table 5. Comparison of Antimicrobial Resistance Rates Between Slow-growing and Fast-growing Nontuberculous Mycobacteria.

Antimicrobials	Slow-growing NTM (n = 143)		Fast-growing NTM (n = 14)		χ^2	p
	Drug resistance	Rate (%)	Drug resistance	Rate (%)		
Amikacin	5	3.50	0	0.00	-	1.000
Amoxicillin/clavulanic acid	129	90.21	14	100.00	-	0.616
Rifampicin	130	90.91	14	100.00	-	0.608
Cefoxitin	129	90.21	9	64.29	-	0.015
Rifabutin	2	1.40	12	85.71	-	0.000
Ciprofloxacin	124	86.71	11	78.57	-	0.418
Clarithromycin	4	2.80	4	28.57	-	0.002
Doxycycline	131	91.61	13	92.86	-	1.000
Imipenem	136	95.10	12	85.71	-	0.185
Linezolid	73	51.05	11	78.57	2.855	0.091
Minocycline	122	85.31	13	92.86	-	0.694
Moxifloxacin	32	22.38	13	92.86	-	0.000
Tigecycline	122	85.31	3	21.43	-	0.000
Tobramycin	37	25.87	11	78.57	-	0.000
Trimethoprim/sulfamethoxazole	16	11.19	12	85.71	-	0.000
Meropenem	128	89.51	13	92.86	-	1.000

"–" indicates Fisher's exact test was used due to expected frequencies < 5, which made the chi-square test inappropriate.

Table 6. Comparison of Antimicrobial Resistance Rates Between *Mycobacterium intracellulare* and *Mycobacterium avium.*

Antimicrobials	Mycobacterium intracellulare (n = 108)		Mycobacterium avium (n = 18)		χ^2	p
	Drug resistance	Rate (%)	Drug resistance	Rate (%)		
Amikacin	5	4.63	0	0.00	–	1.000
Amoxicillin/clavulanic acid	105	97.22	11	61.11	–	0.000
Rifampicin	105	97.22	15	83.33	–	0.038
Cefoxitin	106	98.15	12	66.67	–	0.000
Rifabutin	2	1.85	0	0.00	–	1.000
Ciprofloxacin	98	90.74	13	72.22	–	0.041
Clarithromycin	4	3.70	0	0.00	–	1.000
Doxycycline	103	95.37	13	72.22	–	0.006
Imipenem	106	98.15	15	83.33	–	0.021
Linezolid	62	57.41	7	38.89	2.136	0.144
Minocycline	102	94.44	11	61.11	–	0.000
Moxifloxacin	26	24.07	4	22.22	–	1.000
Tigecycline	104	96.30	9	50.00	–	0.000
Tobramycin	30	27.78	3	16.67	–	0.398
Trimethoprim/sulfamethoxazole	9	8.33	3	16.67	–	0.377
Meropenem	105	97.22	11	61.11	–	0.000

"–" indicates Fisher's exact test was used due to expected frequencies < 5, which made the chi-square test inappropriate.

Table 7. Comparison of Antimicrobial Resistance Rates Between Male and Female.

Antimicrobials	Male (n = 85)		Female (n = 72)		χ^2	p
	Drug resistance	Rate (%)	Drug resistance	Rate (%)		
Amikacin	4	4.71	1	1.39	–	0.376
Amoxicillin/clavulanic acid	73	85.88	70	97.22	6.172	0.013
Rifampicin	77	90.59	67	93.06	0.312	0.576
Cefoxitin	71	83.53	67	93.06	3.325	0.068
Rifabutin	3	3.53	11	15.28	–	0.012
Ciprofloxacin	74	87.06	61	84.72	0.177	0.674
Clarithromycin	5	5.88	3	4.17	–	0.727
Doxycycline	74	87.06	70	97.22	5.302	0.021
Imipenem	78	91.76	70	97.22	–	0.181
Linezolid	41	48.24	44	61.11	2.603	0.107
Minocycline	67	78.82	68	94.44	7.894	0.005
Moxifloxacin	26	30.59	20	27.78	0.149	0.700
Tigecycline	64	75.29	61	84.72	2.135	0.144
Tobramycin	26	30.59	22	30.56	0.000	0.996
Trimethoprim/sulfamethoxazole	14	16.47	14	19.44	0.235	0.628
Meropenem	72	84.71	69	95.83	5.274	0.022

"–" indicates Fisher's exact test was used due to expected frequencies < 5, which made the chi-square test inappropriate.

Significant differences were observed in resistance to amoxicillin/clavulanic acid (85.88% in males vs. 97.22% in females, $p = 0.013$), doxycycline (87.06% vs. 97.22%, $p = 0.021$), minocycline (78.82% vs. 94.44%, $p = 0.005$), and meropenem (84.71% vs. 95.83%, $p = 0.022$). Female patients showed significantly higher resistance rates for these antibiotics compared to male patients. No significant sex-based differences were found for the other agents tested (Table 7).

We also compared antimicrobial resistance between elderly patients (≥ 60 years) and non-elderly patients (< 60 years), as shown in Table 8. Resistance to amoxicillin/clavulanic acid (94.59% in elderly vs. 82.61% in non-elderly, $p = 0.016$) and linezolid (60.36% vs. 39.13%, $p = 0.015$) was significantly higher among elderly patients. These results suggest that age may influence resistance patterns for specific antimicrobials. For the remaining antibiotics, resistance rates did not significantly differ between age groups (Table 8).

Discussion

NTM and tuberculosis share similar clinical manifestations and can both involve the lungs and other organs, with pulmonary disease being the most common presentation [17,18]. Because patients with NTM infection often exhibit symptoms such as cough, sputum production, hemoptysis, and dyspnea, misdiagnosis as tuberculosis is common [19,20]. In addition, the drug-resistance profile of NTM differs substantially from that of *Mycobacterium tuberculosis*. Therefore, accurate species identification and drug-resistance testing are essential for the effective clinical management of both tuberculosis and NTM disease. As a designated tuberculosis treatment center in the region, the institution responsible for this study conducts testing of confirmed tuberculosis cases and mycobacteria-positive isolates referred from surrounding counties and districts. Analysis of the NTM isolation rate and resistance characteristics among patients admitted to this center provides an overall representation of the epidemiological and resistance patterns of NTM in Lishui City.

The results of this study demonstrated that the overall isolation rate of NTM strains from clinical patients in Lishui city was 18.09% during 2023-2024, This rate was lower than the 22.9% reported in the fifth national tuberculosis epidemiological survey in 2010 [21], but higher than the 16.4% reported in Taizhou city [22] and the 7.3% in Wenzhou city [23], and comparable to the 21.4% observed in Hangzhou city [24]. The NTM detection rate in Lishui City increased from 2023 to 2024, a trend consistent with the overall situation in Zhejiang Province [25], the findings in Hangzhou City [24], and other related studies [12,13]. Several factors may account for this rising trend. First, population aging has led to an increased proportion of elderly patients, who are more likely to become a high-risk group for NTM infection. Second, continuous advances in diagnostic technology in recent years have markedly improved the ability to detect NTM. Finally, environmental changes (e.g., increased NTM contamination) and lifestyle shifts may also contribute to the higher detection rate.

NTM detection rates vary considerably across demographic groups. In this study, the isolation rate of NTM among elderly patients was higher than that observed in the overall population. Several factors may explain this finding. First, the higher prevalence of underlying diseases and comorbidities in older adults leads to impaired immune function and reduced resistance to pathogens, thereby increasing

Table 8. Comparison of Antimicrobial Resistance Rates Between Elderly Group (≥ 60 years) and Non-Elderly Group (< 60 years).

Antimicrobials	Non-Elderly Group (<60) (n = 46)		Elderly Group (≥60) (n = 111)		χ^2	p
	Drug resistance	Rate (%)	Drug resistance	Rate (%)		
Amikacin	1	2.17	4	3.60	–	1.000
Amoxicillin/clavulanic acid	38	82.61	105	94.59	5.753	0.016
Rifampicin	42	91.30	102	91.89	0.015	0.903
Cefoxitin	37	80.43	101	90.99	3.407	0.065
Rifabutin	5	10.87	9	8.11	–	0.553
Ciprofloxacin	40	86.96	95	85.59	0.051	0.822
Clarithromycin	2	4.35	6	5.41	–	1.000
Doxycycline	42	91.30	102	91.89	0.015	0.903
Imipenem	42	91.30	106	95.50	–	0.450
Linezolid	18	39.13	67	60.36	5.904	0.015
Minocycline	38	82.61	97	87.39	0.616	0.432
Moxifloxacin	13	28.26	33	29.73	0.034	0.854
Tigecycline	34	73.91	91	81.98	1.305	0.253
Tobramycin	13	28.26	35	31.53	0.164	0.686
Trimethoprim/sulfamethoxazole	8	17.39	20	18.02	0.009	0.926
Meropenem	39	84.78	102	91.89	1.796	0.180

"–" indicates Fisher's exact test was used due to expected frequencies < 5, which made the chi-square test inappropriate.

susceptibility to respiratory tract colonization by NTM. Second, elderly individuals are more likely to seek medical care and undergo relevant examinations, which may contribute to higher detection rates. The study observed a higher NTM detection rate among females, a finding consistent with previous reports [26,27].

The distribution of NTM species exhibits marked geographical variation [1,15,16]. Globally, the *Mycobacterium avium complex* demonstrates a high isolation rate [28,29]. In China, the predominant species are *Mycobacterium intracellulare* and *Mycobacterium abscessus* [30,31]. In this study, nine NTM species were identified, showing distinctive regional characteristics. The detection rate of *Mycobacterium intracellulare* was the highest, 68.79%, a proportion consistent with findings from previous studies in China [32]. *Mycobacterium avium* accounted for 11.46%, and *Mycobacterium chelonae/abscessus* for 8.28%, with these three species together comprising more than 90% of all isolates. In addition, a few rare species, such as *Mycobacterium szulgai/Malmoense* and *Mycobacterium kansasii,* were also isolated. This distribution pattern may be associated with the environmental characteristics of the study area, the sensitivity of the detection method, and the sample base.

The cell walls of NTM are characterized by high hydrophobicity and low permeability, limiting intracellular drug accumulation and resulting in subtherapeutic concentrations that contribute to drug resistance [33–35]. In this study, NTM isolates from Lishui exhibited varying degrees of antimicrobial resistance, with generally high overall resistance rates. The highest resistance was observed for imipenem (94.27%, 148/157), doxycycline (91.72%, 144/157), rifampicin (91.72%, 144/157), amoxicillin/clavulanic acid (91.08%, 143/157), and meropenem (89.81%, 141/157). Resistance patterns differed between slow-growing and fast-growing NTM. Among slow-growing species, resistance rates were highest for imipenem (95.10%, 136/143), doxycycline (91.61%, 131/143), rifampicin (90.91%, 130/143), amoxicillin/clavulanic acid (90.21%, 129/143), and cefoxitin (90.21%, 129/143), while lower resistance rates were noted for rifabutin (1.40%, 2/143), clarithromycin (2.80%, 4/143), amikacin (3.50%, 5/143), trimethoprim/sulfamethoxazole (11.19%, 16/143), and moxifloxacin (22.38%, 32/143). Among fast-growing species, resistance was highest for amoxicillin/clavulanic acid (100.00%, 14/14), rifampicin (100.00%, 14/14), doxycycline (92.86%, 13/14), minocycline (92.86%, 13/14), moxifloxacin (92.86%, 13/14), and meropenem (92.86%, 13/14). In contrast, lower resistance was observed for amikacin (0.00%, 0/14), tigecycline (21.43%, 3/14), and clarithromycin (28.57%, 4/14). The high levels of resistance among NTM strains pose considerable clinical challenges. Patients unresponsive to standard anti-tuberculosis therapy should be evaluated for possible NTM infection, with timely species identification and drug-susceptibility testing to guide individualized treatment. Elderly patients, in particular, often exhibit poor treatment adherence and are prone to underdiagnosis and misdiagnosis, leading to prolonged illness, increased drug tolerance [36,37], and the development of NTM disease resistant to conventional regimens. Therefore, continuous monitoring of NTM species distribution and resistance patterns across different populations is essential.

Subgroup analyses revealed several statistically significant differences in antimicrobial resistance. Fast-growing NTM demonstrated higher resistance rates than slow-growing species to rifabutin (85.71% vs. 1.40%, $p = 0.000$), moxifloxacin (92.86% vs. 22.38%, $p = 0.000$), and trimethoprim/sulfamethoxazole (85.71% vs. 11.19%, $p = 0.000$). In addition, *Mycobacterium intracellulare* exhibited significantly higher resistance than *Mycobacterium avium* to amoxicillin/clavulanic acid (97.22% vs. 61.11%, $p = 0.000$), cefoxitin (98.15% vs. 66.67%, $p = 0.000$), and tigecycline (96.30% vs. 50.00%, $p = 0.000$). Host factors were also associated with resistance variation: female patients showed higher resistance to minocycline (94.44% vs. 78.82%, $p = 0.005$), while elderly patients demonstrated greater resistance to linezolid (60.36% vs. 39.13%, $p = 0.015$). These findings indicate that both bacterial and host factors contribute to heterogeneity in antimicrobial resistance patterns.

Several limitations should be acknowledged. First, all isolates were obtained exclusively from respiratory specimens, which may not accurately reflect resistance patterns in extrapulmonary infections. Second, the limited number of isolates for certain NTM species may have reduced the statistical power of subgroup analyses. Third, the retrospective, single-center design may have introduced selection bias and limited the generalizability of the findings. Future multicenter, prospective studies that include a broader range of specimen types are warranted to validate and extend these results.

Conclusions

In conclusion, the detection rate of NTM among

clinical patients in Lishui City was relatively high and demonstrated an upward trend, with a higher prevalence in females compared with males. The predominant species were *Mycobacterium intracellulare*, *Mycobacterium avium*, and *Mycobacterium chelonae/abscessus*. NTM in this region exhibited distinct geographical characteristics in species distribution and drug-resistance patterns. Early species identification and drug-susceptibility testing are essential for improving clinical diagnosis and treatment, as well as for strengthening the prevention and control of NTM disease in this area.

Funding
This study was supported by the Lishui City Science and Technology Plan Project (No. 2023GYX52); Lishui City Science and Technology Plan Project (No. 2018zdhz10); Science and Technology Department of the State Administration of Traditional Chinese Medicine-Zhejiang Provincial Administration of Traditional Chinese Medicine Co-construction Science and Technology Programme Project (No. GZY-ZJ-KJ-23096) and Zhejiang Province Traditional Chinese Medicine Science and Technology Plan Project (No.2023ZL801).

Availability of data and materials
All data generated or analyzed during this study are included in this article.

Authors' contributions
Henan Xu, Ying Zhang, and Zunjing Zhang designed and supervised the study; Li Lin and Congjuan Lai designed and performed the experiments; Zunjing Zhang, Yutong Zhang, and Jing Guo analysed the data; Henan Xu and Li Lin wrote the manuscript; Zhongda Liu, Ying Zhang, and Zunjing Zhang revised the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate
This study was a retrospective analysis based on anonymized laboratory data obtained from routine clinical diagnostics. Ethical approval was obtained from the Ethics Committee of Lishui City Tuberculosis Sentinel Hospital. Approval No.: Clinical Ethics Review (KY-2024053).

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Conflict of interest
No conflict of interest is declared.

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