Infection and Drug Resistance

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ORIGINAL RESEARCH

Analysis of Clinical Isolation Characteristics of Nontuberculous Mycobacteria and Drug Sensitivity of Rapidly Growing Mycobacteria in the General Hospital of Guangzhou, China

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Purpose: The clinical distribution characteristics of nontuberculous mycobacteria (NTM) in general hospital were explored to guide the clinical diagnosis and treatment of NTM infection.

Methods: Samples with positive mycobacterium culture in the First Affiliated Hospital of Guangzhou Medical University were collected and identified through PCR. Phenotypic drug sensitivity experiments were conducted on 44 *Mycobacteroides abscessus* isolated from clinical departments with broth microdilution method, and *rrl, rrs* and *erm (41)* genes associated with drug resistance were detected.

Results: From September 2020 to July 2023, 314 mycobacterium-positive isolates were separated from patients in the First Affiliated Hospital of Guangzhou Medical University, with 147 (46.8%) NTM isolates were included in our study. The samples were respiratory tract specimens mainly, with 64% bronchoalveolar lavage fluid. Of 144 cases identified, samples were from 133 patients (60 males and 73 females; gender ratio of 0.82:1). NTM was mainly isolated from the people aged 40 and above, especially females (χ^2 = 10.688, P = 0.014). *M. abscessus* (61, 42.36%), *M. intracellulare* (35, 24.31%) were the two most NTMs in this hospital. Clinical strains of *M. abscessus* exhibited high resistance to antibiotics, except for cefoxitin (31.8%), linezolid (25.0%), amikacin (0%), and clarithromycin (18.2%). Among 8 strains of *M. abscessus* with clarithromycin acquired resistance, just 4 strains (50.0%) showed mutations (A2270G, A2271G) in *rrl* gene, but a new mutation (C2750T) was detected in 1 strain. Among 14 strains of *M. abscessus* with clarithromycin-induced resistance, 13 (93.0%) strains had T28 *erm (41)* gene and 1 (7.0%) strain had C28 *erm (41)* gene.

Conclusion: *M. avium–intracellulare* complex was gradually becoming predominant strain in Guangzhou area. The resistant situation of *M. abscessus* in general hospital had shown severe. Potential mutation in *rrl* gene associated with clarithromycin acquired resistance of *M. abscessus* were found, but drug-resistant mechanism remained unclear.

Keywords: nontuberculous mycobacterium, drug resistance, clarithromycin, *Mycobacteroides abscessus*

Introduction

Nontuberculous mycobacteria (NTMs) belong to the genus mycobacterium except, the *Mycobacterium tuberculosis complex* (MTBC, including *Mycobacterium bovis, Mycobacterium africanum*, and *Mycobacterium tuberculosis*) and *Mycobacterium leprae*, which can exist in natural environments, such as soil, rivers, and human settlements.[1] More than 190 types of NTMs have been discovered (http://www.bacterio.net/mycobacterium.html), most of which are parasitic

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bacteria and few are conditional pathogens. NTMs can be divided into two categories by growth rate: rapidly growing mycobacteria (RGMs) and slow-growing mycobacteria (SGMs). Common conditionally pathogenic NTMs in clinical settings include *Mycobacterium avium, Mycobacterium kansasii, Mycobacterium intracellulare, Mycobacteroides abscessus, Mycobacteroides chelonei, Mycobacterium marinum*, and *Mycolicibacterium fortuitum*, which cause lung diseases and are related to infections in the central nervous system, skin, and bone. Patients with acquired immune deficiency syndrome (AIDS) were the high incidence population of NTM disease, and most of them were disseminated NTM disease. *M. chelonei* and *M. fortuitum* were associated with skin, lung, bone, and central nervous system infections. *M. avium* and *M. intracellulare* were the most commonly pathogenic SGM in NTM. And *M. kansasii* was the second most common pathogen causing lung diseases in SGM.[2–4]

Proportion of NTM clinical isolates varies among locations in the Chinese mainland. In South China, the highest clinical isolation rate of NTM is *M. abscessus*.[5,6] The top five NTM strains isolated in Guangzhou area from 2018 to 2021 were *Mycobacterium avium–intracellulare complex, M. chelonei–abscessus complex, M. kansasii, M. fortuitum*, and *Mycobacterium gordonae*,[7] and the highest isolation rate in RGM was obtained in the *M. abscessus* culture. *M. intracellulare* was the most common clinical isolates of NTM in Beijing and Sichuan, China, while the clinical isolation rate of *M. kansasii* in Shanghai, China, was the highest.[8–10] A statistical study on the distribution of isolate strains in NTM lung disease patients in Europe also revealed that *Mycobacterium avium–intracellulare* complex and *M. kansasii* were the two most common pathogens in the European region, with *Mycobacterium avium–intracellulare* complex being the main pathogen in northern Europe and *M. kansasii* being the main pathogen in southern Europe.[11] The isolate quantity of *M. avium* and *M. kansasii* ranked first and second in the NTM of general hospitals in the San Francisco and Virginia, United States.[12,13] NTM infection is often misdiagnosis as *Mycobacterium tuberculosis* infection due to the biological similarities between NTM and MTBC and inadequate experience in the diagnosis and treatment of NTM infections.

Traditional clinical laboratory tests for NTM, including acid-fast staining smear microscopy and Roche solid medium isolation and culture, are still used in nontuberculous-designated medical institutions. Distinguishing among Mycobacteria species is difficult even in mycobacteria culture. Designated medical institutions for tuberculosis prevention and control are difficult to carry out because of their high prices and rigorous operations; nevertheless, advanced molecular diagnostic technologies can directly identify mycobacterium species.[14–16] Based on the different situations of NTM isolation across the country and the fact that clinical laboratory testing methods of NTM in general hospitals were neglected, this study combined with the isolation of NTM at the First Affiliated Hospital of Guangzhou Medical University to improve the data and explore the clinical distribution characteristics of NTM in the general hospital and aimed to guide the clinical diagnosis and drug treatment of NTM infection.

Materials and Methods

Study Design and Setting

This is a survey on the epidemiology and drug-sensitive test of NTM in general hospital in Guangzhou, China. The study was set at the First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China, which was one of the general hospitals in Guangzhou area.

The study sample included 147 patients and their clinical specimens between September 2020 and July 2023. The clinical data of the subjects were collected and analyzed retrospectively, including age, gender, medical history, and clinical diagnosis. Based on gender as the independent variable, this study analyzed statistical differences in age and medical history between different genders. Drug sensitive test. And broth microdilution method and polymerase-chain reaction (PCR) were used in drug-sensitive analysis.

Inclusive Criteria

The inclusive criteria were as follows: (1) Positive results within the reporting period (42 days) for bottles cultured on a mycobacterium liquid culture apparatus (BACTEC MGIT 960, BD, America); (2) smear obtained from the culture solution is positive for acid-fast bacteria; (3) Colloidal gold test for detecting the MPT64 antigen showed negative result.

Identification

The NTM positive liquid culture of BACTEC MGIT 960 was inactivated by using 70% ethanol and ultraviolet ray for 2 hours. Then, nucleic acid from the inactivated bacterial solution was extracted and stored at −20 °C.

Mycobacterium 16S-23S ribosomal deoxyribonucleic acid (rDNA) internal transcribed spacer sequence (ITS) and ribonucleic acid (RNA) polymerase β subunit coding gene (*rpoB*) were used as the target genes for polymerase-chain reaction (PCR). Two pairs of forward and reverse primers were used (Table 1), and the ITS PCR reaction system was 50 μL, which contained a template (2 μL, 20.4–36.3 ng/μL), forward primer and reverse primer (1.8 μL each), 2× Accurate Taq DNA polymerase (25 μL; Accurate Biology), and ddH_2O (20.4 μL). The amplification program was as follows: 5 min at 94 °C; 45 cycles of 45s at 94 °C, 1 min at 55 °C, and 1 min at 72 °C; 10 min at 72 °C. The *rpoB* gene PCR reaction system was 50 μL and contained a template (4 μL), forward primer and reverse primer (2.5 μL each), 2× Accurate Taq DNA polymerase (25 μL), and ddH2O (16 μL). The amplification program was as follows: 5 min at 94 °C; 35 cycles of 45s at 94 °C, 1 min at 52 °C, and 1 min at 72 °C; 10 min at 72 °C. The amplified product was subjected to 130 V and 20 mins of electrophoresis with 2% agarose gel as the carrier.

Primer synthesis and DNA bidirectional seq*uencing* were completed by Shenggong Biotechnology Co., Ltd. (Shanghai, China), and the sequencing primers were the same as the amplification primers. The sequencing results were compared with BLASTN sequences in the NCBI GenBank standard database for the identification of specific bacterial species (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antimicrobial Drug Sensitivity Testing of RGM

The Mycobacterium-positive liquid culture bottles were transferred to Columbia blood agar plates (Autobio Diagnostics, China) and cultured for 5 days in 35 °C in 5% CO_2. Commercial susceptibility test kits (Sensititre RAPMYCOI, ThermoFisher Scientific, America) were used for drug sensitivity tests. The minimum inhibitory concentration (MIC) of 15 antibiotic drugs against the clinical RGM strains were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M24 E3 file (2018),[17] and the breakpoints of drugs were identified using the CLSI M62 ED1 file (2018).[18] The procedures in the operating manual were strictly followed.

We designed primers for the *rrl, erm (41)*, and *rrs* genes were as follows (Table 1), and PCR was performed. The PCR products were then sent to Shenggong Biotechnology Co., Ltd. for sequencing, and BLAST analysis was performed using the NCBI standard database and the standard strain ATCC19977.

Statistical Analysis

Clinical and experimental data were statistically analyzed with IBM SPSS v21.0 software. The comparative analysis of age constituent ratio between age groups used chi-square test or Fisher's exact test. And Wilcoxon rank sum test was used in the comparison of average age between male and female. $P<0.05$ indicated the statistically significant difference.

Table 1 Target Sequences and Primers for PCR Amplification

Primers	Sequence	Length(bp)
rpoB	F 5′-CACGAGGTGCTGGAAGGA-3′ R 5′-CGGGCGATAGCGGAGT-3′	250–750
16S-23S rDNA ITS	F 5′- GAAGTCGTAACAAGG −3′ R 5′- CACGAGGTGCTGGAAGGA −3′	250–750
rrl	F 5′- GAATTGAAACGCTGGCACACT −3′ R 5′- GGTGTCCTACTCTTCCGTTCC −3′	3464
rrs	F 5′-ACCAACGATGGTGTGTCCAT-3′ R 5′-CTTGTCGAACCGCATACCCT-3′	1727
erm (41)	F 5′- GAAGCTGGCAGGCAACG −3′ R 5′- CCGTCGGCACGCAGTA −3′	872

Results

Sample and Clinical Information Analysis

From September 2020 to July 2023, a total of 314 mycobacterium-positive isolates were separated from patients in the First Affiliated Hospital of Guangzhou Medical University, with 147 (46.8%) NTM isolates were included in our study. A total of 144 cases of Mycobacterium infections (68 cases of RGM and 76 cases of SGM) were identified by Sanger sequencing and real-time quantitative PCR (Xiamen Zeesan Biotech, China). The specimens were collected from 85 bronchoalveolar lavage fluids (57.8%), 21 bronchoscopy sputum (14.3%), 12 lung tissues (8.2%), 6 sputum samples (4.1%), 4 pus samples (2.7%), 3 deep sputum samples (2.0%), 3 secretions (2.1%), 2 urine samples (1.3%), and 11 other specimens (7.5%; Figure 1).

A total of 144 NTM samples were collected from 133 patients (60 males, average age of 62.0 ± 18.8 years; 73 females, average age of 55.5 ± 12.7 years) with a gender ratio of 0.82:1 (Table 2). A total of 70 patients were diagnosed with NTM infection, and the proportion of NTM positive patients diagnosed clinically with NTM infection reached 51.9%. There was a statistical difference in the NTM separation rate among different age groups and between genders ($\chi^2 = 10.688$, $P = 0.014$). Male patients were mainly over 60 years old, whereas female patients were mainly 40–60 years old. However, there was no statistical difference in average age between males and females in the Wilcoxon rank sum test ($Z = -1.172$, $P = 0.241$). Patients previously diagnosed with NTM or tuberculosis were more likely to be diagnosed with NTM infections again or clinically isolated with NTM (39/135, 28.9%) (Table 3).

Identification of NTM

A total of 144 cases were identified (Table 4). A total of 61 *M. abscessus* (42.36%), 35 *M. intracellulare* (24.31%), 16 *M avium* (11.11%), 5 *Mycobacterium colombiense* (3.47%), 10 *M. kansasii* (6.94%), 3 *M. fortuitum* (2.08%), and *M. scrofulaceum* (2/1.39%), and *M. phocaicum* (2/1.39%) specimens were identified.

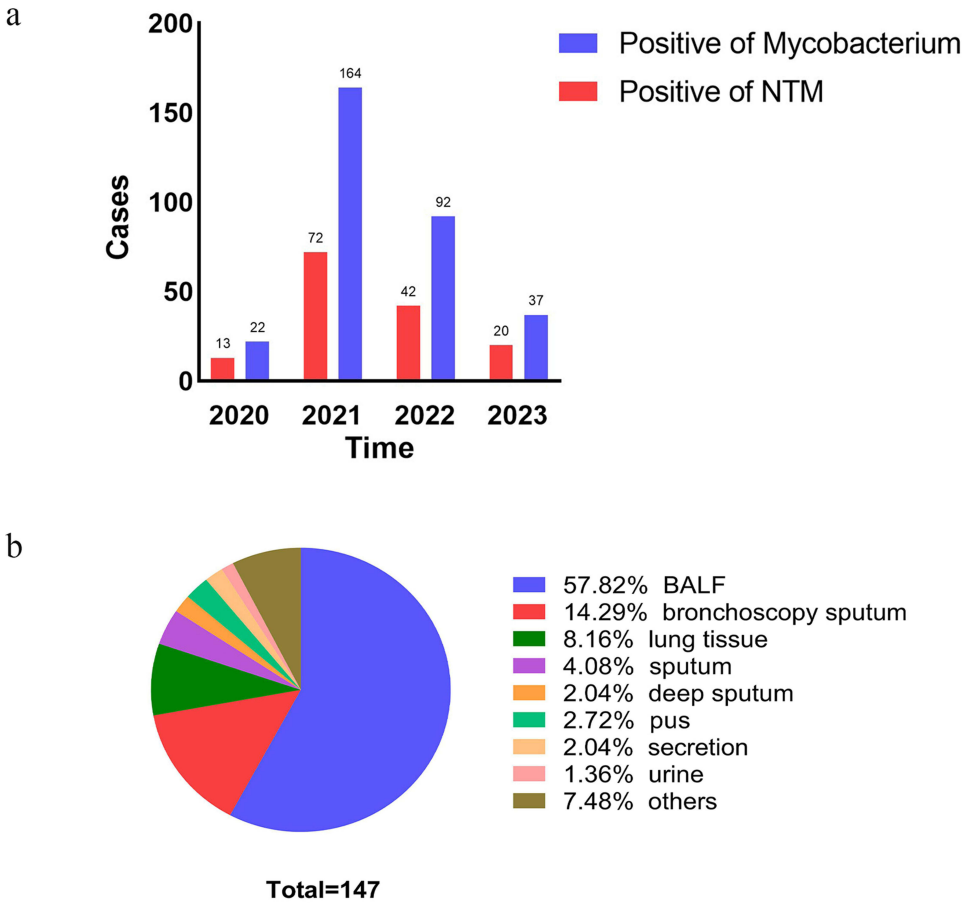

Figure 1 Clinical isolation of Mycobacterium from September 2020 to July 2023 at the First Affiliated Hospital of Guangzhou Medical University (**a**). Sample types of 147 NTM isolates (**b**). NTM, nontuberculous mycobacteria.
Abbreviation: BALF, bronchoalveolar lavage fluid.

Table 2 NTM Positive Cases Between Different Age Groups

Age (Years Old)	Male	Female	Total
≤20	3 (2.26%)	0 (0.00%)	3 (2.26%)
21–40	6 (4.51%)	10 (7.52%)	16 (12.03%)
41–60	16 (12.03%)	35 (26.32%)	51 (38.35%)
>60	35 (26.32%)	28 (21.05%)	63 (47.37%)
Total	60 (45.11%)	73 (54.89%)	133 (100.00%)

Abbreviation: NTM, nontuberculous mycobacteria.

Table 3 Medical History Statistics of NTM Positive Cases

Medical History	Male	Female	Total
Previously diagnosed with NTM infection or tuberculosis	15	24	39
Lung cancer	5	7	12
Lung transplantation	9	1	10
COPD	7	1	8
Smoke	20	0	20
Other tumors/cancers	2	0	2
Accident injury	2	2	4

Abbreviations: NTM, nontuberculous mycobacteria; COPD, chronic obstructive pulmonary disease.

Table 4 Identification of NTM in the First Affiliated Hospital of Guangzhou Medical University

Strains	Number	Percentage (%)	Classification
M. abscessus	61	42.36	RGM
M. intracellulare	35	24.31	SGM
M. avium	16	11.11	SGM
M. colombiense	5	3.47	SGM
M. kansasii	10	6.94	SGM
M. fortuitum	3	2.08	RGM
M. phocaicum	2	1.39	RGM
M. scrofulaceum	2	1.39	SGM
M. senegalense	1	0.69	RGM
M. arupense	1	0.69	SGM
M. szulgai	1	0.69	SGM
M. asiaticum	1	0.69	SGM
M. simiae	1	0.69	SGM
others	5	3.47	/
Total	144	100	

Abbreviations: NTM, nontuberculous mycobacteria; RGM, rapidly growing mycobacteria; SGM, slowly growing mycobacteria.

Results of Antimicrobial Drug Sensitivity Testing

As the NTM with the highest proportion in our study, we completed phenotypic drug sensitivity tests on 44 clinical strains of *M. abscessus*, and detected a total of 7 categories and 15 clinical drugs used for against RGM infection: β-lactam, aminoglycosides, macrolides, quinolones, sulfonamides, tetracyclines, and oxazolidinone (Tables 5 and 6). The drug resistance rates of ciprofloxacin, moxifloxacin, minocycline and doxycycline had reached over 80%; resistance rates of imipenem and tobramycin exceeded 60%; *M. abscessus* shown good sensitivities to cefoxitin, linezolid, amikacin and clarithromycin. However, after extending the cultivation time to 14 days of clarithromycin, rates of susceptible and intermediate were lowered and the increase in drug resistance rate from 18.2% to 50.0%.

Table 5 Results of Drug Sensitivity Tests on 44 Strains of Mycobacteroides abscessus

Drugs	Numbers of Isolates		
	Susceptible	Intermediate	Resistance
CIP	1 (2.3%)	4 (9.1%)	39 (88.6%)
IMI	1 (2.3%)	12 (27.3%)	31 (70.4%)
MXF	2 (4.5%)	4 (9.1%)	38 (86.4%)
TOB	1 (2.3%)	14 (31.8%)	29 (65.9%)
MIN	2 (4.5%)	3 (6.8%)	39 (88.7%)
DOX	3 (6.8%)	4 (9.1%)	37 (84.1%)
SXT	9 (20.5%)	/	35 (79.5%)
FOX	1 (2.3%)	29 (65.9%)	14 (31.8%)
LZD	27 (61.4%)	6 (13.6%)	11 (25.0%)
AMI (IV)	44 (100%)	0	0
CLA (5 days)	35 (79.5%)	1 (2.3%)	8 (18.2%)
CLA (14 days)	20 (45.5%)	2 (4.5%)	22 (50.0%)
FEP	–	–	–
AXO	–	–	–
TGC	–	–	–
AUG2	–	–	–

Abbreviations:CIP, ciprofloxacin; IMI, imipenem; MXF, moxifloxacin; TOB, tobramycin; MIN, minocycline; DOX, doxycycline; SXT, trimethoprim-sulfamethoxazole; FOX, cefoxitin; LZD, linezolid; AMI (IV), amikacin (intravenous); CLA, clarithromycin; FEP, cefepime; AXO, ceftriaxone; TGC, tigecycline; AUG2, amoxicillin/potassium clavulanate 2:1. Cefepime, ceftriaxone, tigecycline, amoxicillin/potassium clavulanate 2:1 had no breakpoints in CLSI M62 ED1 file (2018).[18] Minocycline's breakpoints referred about Nocardia spp. in CLSI M62 ED1 file (2018).[18]

Table 6 Number and Distribution of Mycobacteroides abscessus Drugs Concentration

Drugs	Range	MIC50	MIC90	No. of Strains at Different Drug Concentrations										
				0.12	0.25	0.5	1	2	4	8	16	32	64	128
CIP	0.12–4	>4	>4	0	0	0	1	4	39	/	/	/	/	/
IMI	2–64	64	>64	/	/	/	/	0	1	4	8	4	27	/
MXF	0.25–8	>8	>8	/	0	0	2	4	8	30	/	/	/	/
TOB	1–16	8	16	0	0	0	0	1	14	21	8	0	0	0
MIN	1–8	>8	>8	/	/	/	2	1	2	39	/	/	/	/
DOX	0.12–16	>16	>16	0	0	1	2	2	2	1	36	/	/	/
SXT	0.25/4.75–8/152	8/152	>8/152	/	1	1	3	4	12	23	/	/	/	/
FOX	4–128	64	128	/	/	/	/	/	0	0	1	13	16	14
LZD	1–32	8	32	/	/	/	0	0	11	16	6	11	0	0
AMI	1–64	4	8	0	0	0	0	9	20	13	2	0	/	/
CLA[a]	0.06–16	0.5	16	8	10	6	5	3	1	1	7	/	/	/
CLA[b]	0.06–16	>16	>16	6	4	6	2	1	2	0	22	/	/	/
FEP	1–32	>32	>32	/	/	/	0	0	0	0	2	42	/	/
AXO	4–64	>64	>64	/	/	/	/	/	0	0	0	0	44	/
TGC	0.015–4	0.5	2	1	8	13	13	8	0	/	/	/	/	/
AUG2	2/1–64/32	>64/32	>64/32	/	/	/	/	0	0	0	0	0	44	/

Notes: [a]CLA for 5 days. [b]CLA for 14 days. The drug concentration unit was μg/mL. There were also 3 strains of clarithromycin (5 days) and 1 strain of clarithromycin (14 days) with MIC <0.06 μg/mL.

Abbreviations: CIP, ciprofloxacin; IMI, imipenem; MXF, moxifloxacin; TOB, tobramycin; MIN, minocycline; DOX, doxycycline; SXT, trimethoprim-sulfamethoxazole; FOX, cefoxitin; LZD, linezolid; AMI (IV), amikacin (intravenous); CLA, clarithromycin; FEP, cefepime; AXO, ceftriaxone; TGC, tigecycline; AUG2, amoxicillin/potassium clavulanate 2:1. MIC_{50}, the minimum drug concentration for inhibiting 50% of the experimental strain; MIC_{90}, the minimum drug concentration for inhibiting 90% of the experimental strain.

Clarithromycin Resistance Gene Detection in Mycobacteroides abscessus

Two mechanisms underlie the resistance of *M. abscessus* to clarithromycin. One was a point mutation in the *rrl* gene encoding peptidyltransferase at sites 2058 or 2059 (Escherichia coli numbering). Drug sensitivity results showed resistance within 3–5 days.[19] We sequenced the *rrl* gene related to the clarithromycin-acquired resistance of *M. abscessus* in 8 clinical strains with MIC for clarithromycin of ≥8 μg/mL in 3–5 days. Three strains (No.237–1, 237–2 and 262) showed a mutation in the *rrl* gene at the A2271G site, and 1 strain (No.295) showed the same mutation at the A2270C site. The mutation sites of the *rrl* gene in a drug-resistant strain (No.206) were 837T deletion and C2750T, and the mutation sites of the same gene in 1 strain (No.188) were G17A and insertion of G at 1288 and 1303 sites (Figure 2). 2 drug-resistant strains (Nos.142 and 284) had no mutations in the *rrl* gene. Nucleotides 2016–2625 (*E. coli* numbering) were aligned in the domain V regions in the *rrl* gene, and thus position 2750, which was near the peptidyl transferase center in the No.206 strain, was likely associated with clarithromycin resistance.

The other underlying mechanism was the adenine methylation at site 2058 of the *rrl* gene. This process was attributed to erythromycin methylase encoded by functional *erm (41)* genes.[21] Drug sensitivity results required long-term cultivation to show resistance. The No. 28 base pairs of the *erm (41)* gene exhibit polymorphism with two sequence types: T28 and C28. We also detected the *erm (41)* gene that can induce the drug resistance of *M. abscessus* on 44 clinical isolates in our study. Except for 1 clinical strain that failed to detect the *erm (41)* gene, among the remaining 43 strains, a total of 17 strains (39.5%) had the T28 *erm (41)* gene, consistent with the drug-sensitive phenotype. The MIC of clarithromycin was ≥16 μg/mL when cultivation was extended to 14 days, and 23 sensitive strains (53.5%) of the T28 *erm (41)* gene showed sequence deletions at 61–62 and 156–429 nt sites. Three sensitive strains (7.0%) had the C28 *erm (41)* gene and exhibited sensitive phenotype after 14 days of cultivation.

Moreover, the sensitivity rate of amikacin was 100%, and T1373A, A1375G, C1376T, and G1458T mutations were not found in the *rrs* gene.

Discussion

The clinical isolation rate of NTM from the First Affiliated Hospital of Guangzhou Medical University in 2020–2023 was 46.8%, increasing from 39.13% in 2021 to 45.65% in 2022 (Figure 1). The rate was higher than that in Guangzhou Chest Hospital in 2018–2021 (35.45%).[7] We isolated 68 strains (47.2%) of RGM and 76 strains (52.8%) of SGM, which were consistent with the number in previous research performed in Guangzhou area.[7,20] In Guangzhou, the clinical isolation rate of *M. abscessus* (61/42.36%) was the highest, followed by the clinical isolation

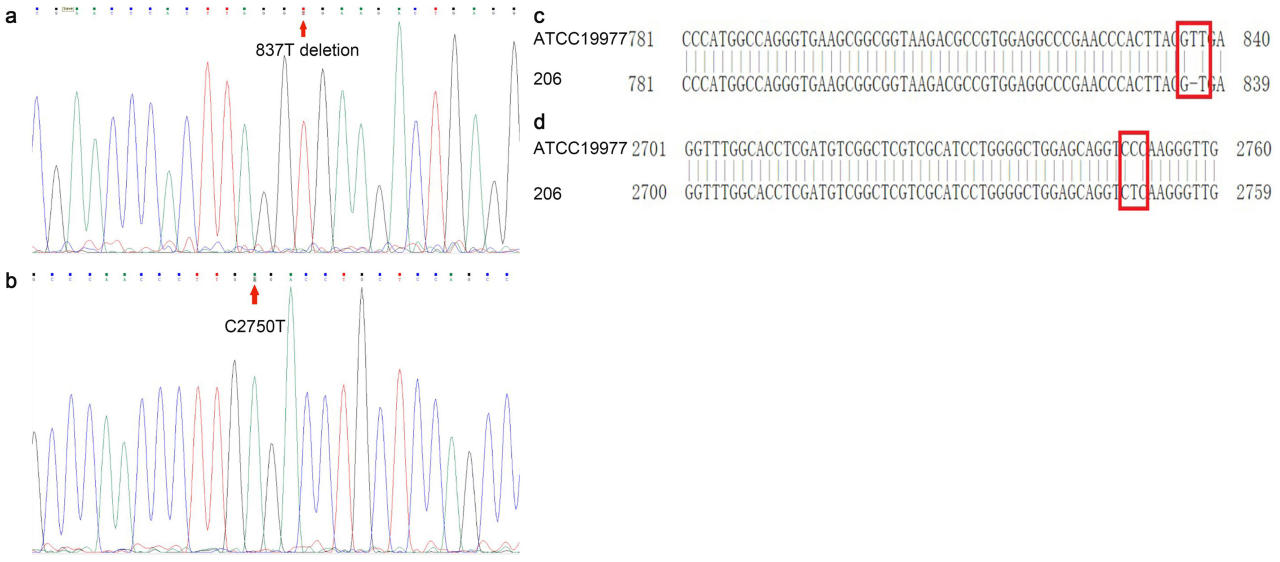

Figure 2 Base deletion, substitution, and insertion in the *rrl* gene of No. 206 (**a** and **b**). Alignment with *rrl* gene of ATCC19977 and No. 206 (**c** and **d**).

rates of *M. intracellulare* (35/24.31%), *M. avium* (16/11.11%), and *M. colombiense* (5/3.47%). These rates are inconsistent with those reported in Nanjing, Chengdu, and Shanghai.[10,22,23] Compared with the clinical isolates of NTM in Guangzhou 5 years ago,[24] the *M. avium complex* including *M. avium, M. intracellulare*, and *M. colombiense* isolates was higher in number, and the proportion of *M. intracellulare* and *M. colombiense* has considerably increased. Therefore, the epidemic status and clinical distribution characteristics of the *M. avium complex* in Guangzhou in the past 5 years should be examined.

In Guangzhou, NTMs were common in middle-aged and elderly people, especially in women, unlike in Sichuan and Chongqing.[25–27] Patients who had previously been diagnosed with tuberculosis or NTM infections were more likely to possess NTMs because of multidrug resistance of NTMs, especially *M. abscessus* complex, to first-line antituberculosis drugs.[28] NTMs had high resistance to most first-line antituberculosis drugs, and common pathogenic strains of *M. intracellulare, M. avium*, and *M. chelonei* exhibit multidrug-resistant characteristics and are resistant to at least eight antibiotics.[29,30] Thus, drugs were usually ineffective against NTM infections, which eventually recur. Drug sensitivity tests should be conducted timely on patients with multidrug-resistance NTMs.

We identified 44 clinical RGM strains through a phenotypic drug sensitivity test. *M. abscessus* was less sensitive to conventional anti-NTM infections drugs, and the sensitivity of *M. abscessus* isolate strains from Guangzhou to linezolid was only 61.4%, and the resistance rate was up to 25.0%, which was much higher than that reported in the Beijing general hospital from 2012 to 2015 (9.7%).[31] This difference may be attributed to two reasons. First, linezolid was commonly used to treat bacterial lung infections, especially those caused by methicillin-resistant *Staphylococcus aureus*. Patients often come into contact with this drug and are prone to develop drug-resistant strains.[32] Second, linezolid could be used in the treatment of drug-resistant pulmonary tuberculosis disease and extrapulmonary tuberculosis disease,[33] and most strains in this study were isolated from patients who had been infected with M. tuberculosis or NTMs, indicating that previous antibiotic treatment was ineffective and may have increased linezolid resistance. Fortunately, first-line drugs for treating NTM infections, such as amikacin and clarithromycin, showed low resistance rates. However, the resistance rate of *M. abscessus* for clarithromycin reached 50.0% at prolonged cultivation time. Identifying clarithromycin resistance targets in *M. abscessus* is more important than identifying amikacin resistance targets, and strains that produce acquired and inducible resistance are needed for the identification of resistance genes and genotypes. In this study, we discovered a new mutation in the *rrl* gene. This mutation was related to the clarithromycin resistance of *M. abscessus* in one clinical strain,[34] and the proportion of common mutation sites was 50.0% (4/8). Other strains without resistance mutation sites exhibited different resistance mechanisms that need to be further explored. The MIC_{50}s and MIC_{90}s of β-lactam drugs were higher than those of other antibiotics in *M. abscessus* possibly because of the highly active β-lactamase (Bla_Mab) encoded by the MAB_2875 gene and the thick cell wall of *M. abscessus* with a large amount of lipids.[35,36] Furthermore, although tigecycline had no drug breakpoints in CLSI guidelines, we determined the MICs for *M. abscessus*, which can further improve the experimental data for reference.

However, samples were collected only from the First Affiliated Hospital of Guangzhou Medical University and the samples from other general hospitals were not included in the study, which cannot fully reflect the NTM isolation in other general hospitals in Guangzhou. Moreover, the MPT64 antigen test results of the specimens included in this study were all negative, but the MPT64 antigen of *Mycobacterium tuberculosis* complex was positive. So, the possibility of mixed infection of *Mycobacterium tuberculosis* complex and NTM was not considered. For this study, it was still necessary to expand the sample size, improve the research data required for the analysis of clinical isolation characteristics of NTM in Guangzhou general hospitals, and systematically explain the prevalence of NTM in the Guangzhou area.

Conclusion

The clinical isolation rates of NTMs in the First Affiliated Hospital of Guangzhou Medical University were higher than the Guangzhou Chest Hospital. NTMs might be isolated more easily in female patients and people who had been diagnosed with tuberculosis or NTM infections. The isolation rate of *M. avium-intracellulare* complex had shown a significant upward trend, gradually becoming a dominant strain in Guangzhou. The drug resistance rates of

M. abscessus from the general hospital in Guangzhou had increased, especially to linezolid and clarithromycin. Common drug-resistant mutation sites in the *rrl* gene accounted for a small proportion of the resistance.

Ethics

This study had been reviewed by the Medical Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University and had obtained ethical permission, with ethics number 2022121.

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Disclosure

The authors report no conflicts of interest in this work.

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