Research article

Prevalence and antimicrobial susceptibility pattern of *Mycobacterium abscessus* complex isolates in Chongqing, Southwest China

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ABSTRACT

Objectives: To investigate the prevalence of *Mycobacterium abscessus* complex (MABC), drug resistance characteristics, and the relationship between clarithromycin (CLA) susceptibility and MABC genotype in Chongqing, China.

Methods: A total of 434 NTM patient isolates were collected between October 2018 and October 2019. Isolates confirmed to be non-tuberculous mycobacteria (NTM) were tested for minimal inhibitory concentrations of antimicrobial agents. In addition, *rrl* and *erm(41)* gene sequences were used to analyze the acquired macrolide resistance and inducible macrolide resistance.

Results: Overall, 17 different NTM species were detected, of which *M. abscessus* (22.6 %, 91/403) was most prevalent. Amikacin, CLA, azithromycin and cefoxitin exhibited potent activities against MABC organisms, but no significant differences were observed in drug resistance rates between *M. abscessus* and *M. massiliense* ($P > 0.05$). On day 3 of culture, the acquired resistance rate against CLA was 7.4 % (9/121). Of 41 MABC isolates with inducible CLA resistant, 95.1 % (39/41) isolates belonged to the *erm(41)* T28 sequevar, while the remaining 4.9 % (2/41) possessed the *M. massiliense* genotype. All *erm(41)* C28 sequevar isolates were sensitive to CLA on day3 and day 14 of culture. Meanwhile, of the 5 *erm(41)* T28 isolates with acquired resistance, all possessed *rrl* 2058/2059 mutations, including 3 isolates with A2058C mutation and 2 isolates with A2059G mutation. While 2 of the 4 *M. massiliense* isolates with acquired resistance possessed the A2059G mutation, and one isolate possessed the A2058G mutation.

Conclusion: Erm(41) and *rrl* gene could serve as useful markers for predicting macrolide susceptibility of MABC complex isolates.

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1. Introduction

The incidence of non-tuberculous mycobacteria (NTM) infections has increased worldwide concurrently with increases in numbers of at-risk populations, such as immunocompromised hosts, the elderly, and patients with cystic fibrosis [1,2]. In China, the prevalence of NTM infections has been rising relative to infections caused by *Mycobacterium tuberculosis,* such that NTM species currently account for approximately one-quarter of mycobacterial patient isolates according to national population-based data [3,4]. Although similar clinical pulmonary manifestations accompany different types of mycobacterial infections, treatment regimens differ greatly. Therefore, accurate identification of NTM species is extremely important prior to treatment administration [5,6].

Of infections caused by NTM species, those infected by *Mycobacterium abscessus* complex (MABC) are especially difficult to treat, due to the fact that these organisms are resistant to most first-line antibiotics and have natural resistance to anti-tuberculosis agents [7, 8]. Currently, macrolides serve as key drugs used to treat MABC infections [9], although macrolide susceptibility varies with *M. abscessus* subspecies (*M. abscessus*, *M. massiliense*, *M. bolletii*), due to two confirmed distinct molecular mechanisms underlying observed clarithromycin (CLA) resistance, acquired and inducible resistance. Acquired macrolide resistance results from point mutations at positions 2058/2059 of the 23S rRNA (*rrl*) gene, while inducible resistance results from expression of an erythromycin ribosomal methylase encoded by an intact *erm(41)* gene, as found in *M. bolletii* and *M. abscessus* subspecies of MABC [10]. However, T/C polymorphisms at position 28 in the *M. abscessus erm(41)* gene result in either inducible resistance (T28) or intrinsic susceptibility to CLA (C28). Notably, *M. massiliense* isolates usually possess a truncated, nonfunctional *erm(41)* gene resulting from a 274-bp deletion that is responsible for the macrolide susceptible phenotype [11–13].

This study was undertaken to investigate MABC prevalence, drug resistance phenotypes, and the relationship between CLA susceptibility and MABC genotypes in Chongqing, the only municipal city in Southwest China with a high incidence of tuberculosis (TB).

2. Materials and methods

2.1. Bacterial strains

A total of 434 NTM patient isolates identified by P-nitrobenzoic acid medium (PNB) were collected in this study between October 2018 and October 2019 from Chongqing Municipality, China. They were further confirmed to be NTM species using multilocus sequence analysis based on 16S rRNA, *hsp65*, *rpoB*, and 16S–23S rRNA internal transcribed spacer (*ITS*) sequences. After excluding 20 *Cutibacterium acnes*, 4 *Gordonia bronchialis*, 1 *Mycobacterium tuberculosis*, 1 *Nocardia cyriacigeorgica*, 1 *Nocardia asteroids* and 4 failed to be sequenced, 403 NTM isolates were included for further analysis [4]. Patient demographic information associated with the isolates was obtained from patient questionnaires.

2.2. MIC assays

Minimal inhibitory concentrations (MICs) of antimicrobial agents were determined using a broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Bacterial suspensions were prepared from subcultures grown in Löwenstein-Jensen medium diluted in saline solution to a density equivalent to that of a 0.5-McFarland standard. Next, bacteria were cultured in cation-adjusted Mueller Hinton (CAMHB) broth (pH 7.3–7.4) to a final inoculum density of approximately 5×10^5 CFU/mL. Therefore, 100 μL of bacterial suspension was added to wells of 96-well microtiter plates containing successive two-fold dilutions of the various antimicrobial agents. After plates were incubated at 37 °C for 3 days, susceptibility to antimicrobial agents was assessed by visual inspection using an inverted mirror, based on breakpoints as indicated in Table 1. Two different breakpoints were used to interpret CLA resistance of isolates after culture: for acquired CLA resistance, the breakpoint was defined as MIC ≥8 mg/L after 3-day culture; for induced resistance, the breakpoint was defined as MIC ≤4 mg/L after 3-day culture and MIC ≥8 mg/L after 14-day culture.

2.3. DNA amplification and sequencing

Crude DNA preparations were obtained from all isolates by heating suspensions of mycobacteria in Tris-EDTA buffer at 100 °C for

Table 1
The antimycobacterial agents and breakpoints.

Antibiotics		MIC range (μg/ml)		
		Sensitive	Intermediate	Resistant
Clarithromycin	CLA	≤2	4	≥8
Azithromycin	AZM	≤16	–	≥32
Amikacin	AMK	≤16	32	≥64
Cefoxitin	FOX	≤16	32–64	≥128
Imipenem	IMP	≤4	8–16	≥32
Linezolid	LZD	≤8	16	≥32
Moxifloxacin	MFX	≤1	2	≥4
Gatifloxacin	GFX	≤1	2	≥4
Levofloxacin	LFX	≤1	2	≥4

15–20 min followed by removal of bacterial debris via centrifugation at 10,000 rpm for 10 min. DNA regions corresponding to *rrl* and *erm(41)* gene were amplified with primer Forward 5-CCTGCACGAATGCGGTAACG-3 and Reverse R 5-CACCAGAGGTTCGTCCGTC-3, and the *erm(41)* gene segments were amplified with primers Forward 5-ACGTTGGATCCGAGCGCCGTCACAAGATGCACA-3 and Reverse 5-GCGAGAAGCTTGACTTCCCCGCACCGATTCCAC-3 [10]. PCR amplification products were sent to Tsingke Co. (Beijing, China) for DNA sequencing. DNA sequences were aligned with homologous sequences of MABC standard strains using BioEdit Sequence Alignment Editor 7.1.3 (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

2.4. Statistical analysis

Chi-square tests were performed to compare proportions of resistant isolates between *M. abscessus and M. massiliense*, MABC subspecies using SPSS 16.0 (SPSS Inc., Chicago, IL). Differences were considered significant for *P*- values < 0.05.

3. Results

3.1. Proportion of different NTM species

Of 403 NTM isolates, the most prevalent NTM species were *M. abscessus* (22.6 %, 91/403), followed by *M. intracellulare* (21.1 %, 85/403), *M. fortuitum* (13.6 %, 55/403), *M. massiliense* (9.7 %, 39/403), *M. avium* (7.7 %, 31/403), *M. gordonae* (7.2 %, 29/403), and *M. kansasii* (6.5 %, 26/403), which together accounted for 88.3 % of NTM isolates studied in this work (Fig. 1).

3.2. Demographic and clinical features of pulmonary Mycobacterium abscessus complex diseases

Excluding 9 isolates with no drug susceptibility testing results available, 121 MABC isolates were included for further analysis. Demographic and clinical characteristics of pulmonary *M. abscessus* complex patients are summarized in Table 2. The average age was 43.5 years and 85 (70.2 %, 85/121) of these patients were male. Furthermore, 74 (61.2 %, 74/121) of these patients reported histories of active TB disease, and 77 patients (63.6 %, 77/121) exhibited cough. Surprisingly, only 29.8 % (36/121) of smears of MABC isolates smears were positive for acid-fast bacilli. Pulmonary radiographic findings indicated that 31 MABC-infected patients (25.6 %) harboured fibrocavitary lesions and 50 (41.3 %, 50/121) patients were afflicted with nodular bronchiectasis.

3.3. In vitro drug susceptibility profiles of M. abscessus complex

As shown in Table 3, of all antimicrobial agents tested herein, amikacin (AMK) was most effective against MABC organisms, with all isolates found to be sensitive to AMK. Clarithromycin (CLA), azithromycin (AZM), and cefoxitin (FOX) also exhibited potent activities
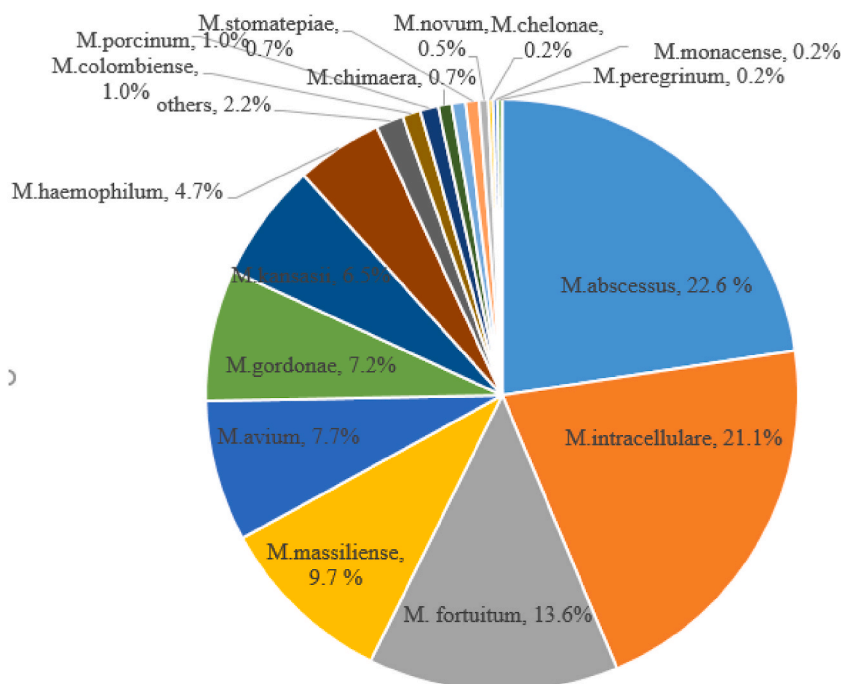

Fig. 1. Distribution of nontuberculous mycobacteria species isolated from pulmonary NTM patients in Southwest China.

Table 2
Demographic and clinical characteristics of *Mycobacteria abscessus* complex infections in this study.

Characteristics	No. of subjects (%)	χ^2	P
Gender			
Male	85 (70.2)	39.686	<0.01
Female	36 (29.8)		
Average age (years)	43.5		
Treatment history			
Previously treated case	89 (73.6)	53.702	<0.01
New case	32 (26.4)		
Comorbidities			
TB history	74 (61.2)	233.885	<0.01
COPD	7 (5.8)		
Bronchiectasis	25 (20.7)		
Malignant diseases	3 (2.5)		
Diabetes mellitus	10 (8.3)		
HIV	3 (2.5)		
Clinical presentations			
Cough	77 (63.6)	240.963	<0.01
Haemoptysis	2 (1.7)		
Fever	2 (1.7)		
Chest distress	3 (2.5)		
Laboratory investigations			
AFB smear (+)	36 (29.8)	100.474	<0.01
Culture (+)	112 (92.6)		
Radiographic inspection			
Fibrocavitary lesions	31 (25.6)	9.438	0.024
Nodular bronchiectasis	50 (41.3)		
Combination	31 (25.6)		
Others	40 (33.1)		

Table 3
Comparison of in vitro drug susceptibility profiles between *M. abscessus* and *M. massiliense* isolates.

Antibiotics	Species	MIC (µg/ml)			No. of resistant isolates (%)			P-value
		Range	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant	
CLA	*M. abscessus*	0.0625–128	0.25	4	73 (89.0)	4 (4.9)	5 (6.1)	0.415
	M. massiliense	0.0625–128	0.0625	0.25	34 (87.2)	1 (2.6)	4 (10.3)	
AZM	*M. abscessus*	0.0625–128	2	32	73 (89.0)	–	9 (11.0)	0.572
	M. massiliense	0.0625–128	0.25	16	36 (92.3)	–	3 (7.7)	
AMK	*M. abscessus*	0.0625–128	4	8	81 (98.8)	1 (1.2)	0 (0.0)	–
	M. massiliense	0.0625–128	4	8	37 (94.9)	2 (5.1)	0 (0.0)	
FOX	*M. abscessus*	0.0625–128	32	64	8 (9.8)	70 (85.4)	4 (4.9)	0.120
	M. massiliense	0.0625–128	64	128	4 (10.3)	30 (76.9)	5 (12.8)	
IPM	*M. abscessus*	0.0625–128	64	128	4 (4.9)	2 (2.4)	76 (92.7)	0.651
	M. massiliense	0.0625–128	64	256	1 (2.6)	1 (2.6)	37 (94.9)	
LZD	*M. abscessus*	0.0625–128	16	32	12 (14.6)	31 (37.8)	39 (47.6)	0.500
	M. massiliense	0.0625–128	16	32	6 (15.4)	17 (45.6)	16 (41.0)	
MFX	*M. abscessus*	0.0625–128	8	16	5 (6.1)	6 (7.3)	71 (86.6)	0.406
	M. massiliense	0.0625–128	8	32	2 (5.1)	3 (7.7)	34 (87.2)	
GFX	*M. abscessus*	0.0625–128	4	8	5 (6.1)	7 (8.5)	70 (85.4)	0.788
	M. massiliense	0.0625–128	8	16	3 (7.7)	2 (5.1)	34 (87.2)	
LFX	*M. abscessus*	0.0625–128	32	64	2 (2.4)	2 (2.4)	78 (95.1)	0.161
	M. massiliense	0.0625–128	16	64	0 (0.0)	0 (0.0)	39 (100.0)	
TIG	*M. abscessus*	0.0625–128	0.5	0.5	–	–	–	–
	M. massiliense	0.0625–128	0.5	1	–	–	–	
BDQ	*M. abscessus*	0.016–32	0.0625	32	–	–	–	–
	M. massiliense	0.016–32	0.0625	32	–	–	–	
CFZ	*M. abscessus*	0.016–32	0.0625	4	–	–	–	–
	M. massiliense	0.016–32	0.0625	0.25	–	–	–	
DLM	*M. abscessus*	0.016–32	>32	>32	–	–	–	–
	M. massiliense	0.016–32	>32	>32	–	–	–	
PA-824	*M. abscessus*	0.016–32	>32	>32	–	–	–	–
	M. massiliense	0.016–32	>32	>32	–	–	–	

against MABC organisms, since percentages of resistant strains for each MABC subspecies were only 6.1 %, 11 %, 4.9 % for *M. abscessus*, and 10.3 %, 7.7 %, 12.8 % for *M. massiliense*, respectively; statistical analysis revealed that differences in resistant rates for a given drug between the two MABC subspecies were not significant ($P > 0.05$).

Table 4
Incubation time on the MIC values and resistant rate of CLA antibiotic for *M. abscessus* subtype, *erm(41)* sequevar.

Subtypes	Number of isolates (n)	Incubation time (days)	CLA MIC (µg/ml)												Number of resistantIsolates (%)
			≤0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	≥128	
abscessus T28	60	3	8	4	8	12	16	3	4	1	1	1	0	2	5 (8.3)
		14	1	1	3	1	3	2	5	3	1	4	4	32	44 (73.3)
abscessus C28	22	3	13	6	2	1	0	0	0	0	0	0	0	0	0 (0.0)
		14	6	8	5	2	0	0	1	0	0	0	0	0	0 (0.0)
massiliense	39	3	25	4	1	0	4	0	1	1	1	0	0	2	4 (10.3)
		14	22	3	3	2	2	1	0	2	0	0	0	4	6 (15.4)
All subtypes	121	3	46	14	11	12	20	4	5	2	2	1	0	4	9 (7.4)
		14	29	12	11	5	5	3	6	5	1	4	4	36	50 (41.3)

3.4. Clarithromycin resistant mechanism

Of 121 MABC isolates, 82 belonged to the subspecies *M. abscessus,* of which 60 belonged to the *erm(41)* T28 sequevar and 22 belonged to the *erm(41)* C28 sequevar, while the remaining 39 isolates belonged to subspecies *M. massiliense* (based on a truncated *erm (41)* gene).

CLA MIC values stratified according to MABC subspecies, *erm(41)* sequevar, and time of incubation are shown in Table 4. On day 3 of culture, 9 *M. abscessus* isolates were resistant to CLA, for an acquired resistance rate of 7.4 % (9/121), as compared to 50 *M. abscessus* complex isolates with CLA resistance that were detected on culture day 14. Of 41 MABC isolates with inducible CLA resistance, 95.1 % (39/41) belonged to the *erm(41)* T28 sequevar and 4.9 % (2/41) were identified as *M. massiliense;* all organisms belonging to the *erm (41)* C28 sequevar exhibited sensitivity to CLA on both day 3 and day 14 of culture.

Of 9 MABC isolates exhibiting acquired CLA resistance, 5 belonged to the *erm(41)* T28 sequevar and the remaining 4 belonged to *M. massiliense*. Of the 5 *erm(41)* T28 sequevar isolates, all possessed *rrl* 2058/2059 mutations, including 3 isolates with A2058C mutation, 2 isolates with A2059G mutation. While 2 of 4 *M. massiliense* isolates harboured the A2059G mutation and one isolate possessed the A2058G mutation (Table 5).

4. Discussion

Pulmonary NTM disease has emerged in recent years as an increasingly serious public health concern worldwide [14,15]. Among pulmonary infections associated with rapidly growing mycobacterial species, 80 % are caused by MABC organisms [13,16,17]. Indeed, in this study MABC was the predominant NTM species isolated from pulmonary NTM patients in Chongqing, as consistent with results obtained by Pang et al. and Zhang et al. [6,18], while contradicting results obtained in northern China showing *Mycobacterium intracellulare* as the predominant NTM species in that region [1,18,19]. This observed geographical diversity of NTM isolates may reflect climate variations across China. Notably, in this study 70.2 % of patients yielding NTM isolates were men, as consistent with other reports from Chongqing [6], an observation that may reflect differences between males and females with regard to immune responses against mycobacteria [15]. Moreover, we found that patients with TB histories were at greater risk of contracting pulmonary MABC-induced disease, a finding that may be attributed to severe pulmonary structural damage resulting from effects of previous active TB disease [20]. Cough, the most common symptom experienced by patients with MABC associated pulmonary disease, was clinically indistinguishable from cough associated with TB. This similarity has often led to misdiagnosis of pulmonary *M. abscessus* infections as TB, thus underscoring the importance of accurate diagnosis.

Analysis of the resistance spectrum of MABC isolates studied here revealed that AMK, CLA, AZM and FOX agents with greatest antimicrobial activities were associated with isolate susceptibility rates of > 85 %, as reported in another study conducted in Shanghai [21]. Notably, in this study all MABC isolates were susceptible to AMK, in accordance with several published studies reporting overall AMK susceptibility rates of >90 % [21,22]. By contrast, fluoroquinolone antibiotics, such as GFX, LFX, and MFX, were shown here to possess unsatisfactory antimicrobial activities against MABC isolates, as consistent with reports of fluoroquinolones resistance rates across China of >85 % that may stem from overuse of these drugs [22,23]. Moreover, newer antimicrobial drugs (DLM and PA-824) studied in this work also exhibited unsatisfactory antimicrobial against MABC isolates, as reflected by high observed drug resistance rates approaching 100.0 %. However, our results should be confirmed through additional studies based on other MABC isolates obtained across China. According to a previous research in Shanghai, the resistance rates to clarithromycin and doxycycline in isolates of *M. abscessus* were significantly higher than those in isolates of *M. massiliense* ($P < 0.05$), whereas here no significant differences were observed in drug resistance rates between MABC subspecies *M. abscessus* and *M. massiliense*, with contradictory possibly due to differences in isolates or patient treatment histories across studies [21].

Macrolide-based antibiotics are regarded as the cornerstone of treatment for MABC infections. In our study, the acquired CLA resistance rate (on day 3) for isolates of MABC subspecies *M. abscessus* was 6.1 %, a rate similar to that reported in another study conducted in China (8.1 %). However, this rate was higher than that reported in the USA (2.51 %), although it was lower than rates reported in France (9.09 %) and South Korea (15.84 %) [11,24,25]. However, the rate of CLA resistant isolates of *M. abscessus* (100 %) and *M. massiliense* (75 %) harboring *rrl* 2058/2059 mutations is significantly higher than those observed by other groups from China [11,21]. The contradictory results possibly attribute to geographic diversity and differences in clinical use of antibiotics. Furthermore, a high rate of inducible resistance was observed for *erm(41)* T28 sequevar isolates, as consistent with reported results of other studies, often resulting in treatment failure [25–27]. Notably, in this work one *M. massiliense* isolate showed inducible resistance to CLA in the absence of detectable *rrl* 2058/2059 point mutations that mechanistically may have been due to mutations leading to altered 50S ribosomal subunit structure [7]. Meanwhile, all *M. massiliense* isolates belonging to the *erm(41)* C28 sequevar were sensitive to CLA. Taken together, these results indicate that *erm(41)* may be useful for predicting drug susceptibility profiles of MABC subspecies.

5. Conclusion

In conclusion, our results suggest that MABC organisms are the predominant cause of NTM lung infections in Chongqing. Antibiotics AMK, CLA, AZM, and FOX exerted potent inhibitory activity against MABC organisms. Importantly, *erm(41)* and *rrl* genes are promising markers for use in predicting MABC macrolide susceptibility.

Table 5

Characterization of the *M. abscessus* clinical isolates with phenotypic resistance to CLA.

Genotype	*rrl* mut				*rrl* mut/3d resistant (%)
	A2058C	A2058G	A2059G	Total number	
erm(41)T28	3	0	2	5	5/5 (100)
M.massiliense	0	1	2	3	3/4 (75)

Ethics statement

This study was approved by the Ethics Committee of the Chongqing Tuberculosis Control Institute (KY201801). Informed consent was obtained from all subjects involved in the study.

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Data availability statement

Data will be made available on request from the corresponding author.

CRediT authorship contribution statement

Yan Hu: Writing – original draft. **Tongxin Li:** Methodology, Data curation. **Wenguo Liu:** Validation, Methodology. **Damian Zhu:** Methodology, Data curation. **Xin Feng:** Investigation, Data curation. **Yaokai Chen:** Writing – review & editing, Supervision, Conceptualization. **Huiwen Zheng:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Yan Hu reports a relationship with Chongqing Municipal Science and Technology Projects that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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