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MECHANISMS OF RESISTANCE

Genetic Correlation of Antibiotic Susceptibility and Resistance Genotyping for the *Mycobacterium abscessus* Group

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ABSTRACT Treatment efficacy of *Mycobacterium abscessus* infections depends on bacterial genotype. Here, the relationship between genotype, as determined by sequence analysis, and antibiotic resistance phenotype was analyzed. The results demonstrate that *M. abscessus* genotype characteristics, including *erm*(41) sequevar and mutations of *rrl* and *rrs*, are predictive of clarithromycin and amikacin resistance.

KEYWORDS *Mycobacterium abscessus*, genotype, resistance

Mycobacterium abscessus, the most common rapidly growing mycobacterium (RGM) isolated from patients with chronic pulmonary infections, accounts for 80% of RGM isolates (1). Treatment of *M. abscessus* infections is complicated because of its innate multidrug resistance that can render even combination antibiotic treatment ineffective (2). Until recently, clarithromycin (CLA) and azithromycin (AZM) formed the cornerstone of antimicrobial chemotherapy of pulmonary *M. abscessus* infections (3). However, two molecular resistance mechanisms underlying CLA resistance were recently described. First, point mutations at position 2058/2059 of the 23S rRNA (*rrl*) gene confer acquired resistance to CLA (4). Second, an intact *erm*(41) T28 sequevar, characterized by a C to T substitution at position 28, confers inducible CLA resistance (4). Notably, *M. abscessus* subsp. *massiliense* (*M. massiliense*) usually possesses a truncated, nonfunctional *erm*(41) gene due to a 274-bp deletion in the gene. In contrast, the *M. abscessus* subsp. *abscessus erm*(41) C28 sequevar, with a C at position 28 of *erm*(41), exhibits intrinsic susceptibility to CLA (5–7). Because of emerging CLA resistance, amikacin (AMK) has commonly been used as an alternative treatment. However, mutations of nucleotide 1408 of the *rrs* gene that have been correlated with AMK resistance have been observed (7). Consequently, because the effectiveness of therapeutic regimens for *M. abscessus* infections varies with bacterial genotype, this study was conducted to correlate *M. abscessus* genotype with antibiotic susceptibility.

A total of 385 isolates were collected from Guangzhou Chest Hospital in southern China and Shanghai Pulmonary Hospital in eastern China from January 2012 to December 2014. All isolates were subcultured on Löwenstein-Jensen medium followed by genomic DNA preparation, as previously reported (8). Isolates were then characterized further by using multilocus sequence analysis of various genes, including 16S rRNA, heat shock protein 65 (*hsp65*), the β-subunit of bacterial RNA polymerase (*rpoB*), and the 16S to 23S rRNA internal transcribed spacer (ITS) sequence. Meanwhile, MICs of 18 of the most common antibiotics used clinically to treat *M. abscessus* infections were determined by use of the recommended standard broth microdilution method (9). The MIC, defined here as the lowest concentration of antibiotic that inhibits visible growth of mycobacteria, was monitored on days 3, 7, and 14 of incubation. Susceptibility breakpoints for each antibiotic are shown in Table S1 in the supplemental material.

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TABLE 1 Incubation time on the MIC values and resistant rate of CLA antibiotic for *M. abscessus* subtype, *erm(41)* sequevar

M. abscessus subtype	No. of isolates	Incubation time (days)	No. of isolates at CLA MIC (μg/ml) of:											No. of resistant isolates (%)
			0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	
T28	185	3	19	31	27	28	32	27	10	4	1	1	5	11 (5.9)
		7	5	9	6	8	13	22	24	25	21	17	35	98 (53.0)
		14	0	0	0	1	1	0	1	16	19	32	115	182 (98.4)
C28	33	3	10	9	6	2	3	0	0	1	0	0	2	3 (9.1)
		7	8	10	7	1	3	0	1	1	0	1	1	3 (9.1)
		14	6	7	7	4	3	3	0	0	1	0	2	3 (9.1)
massiliense	163	3	51	43	28	12	4	4	4	5	3	2	7	17 (10.4)
		7	43	37	24	17	10	10	5	4	3	2	8	17 (10.4)
		14	29	20	17	24	22	27	5	2	1	4	12	19 (11.7)
All subtypes	381	3	80	83	61	42	39	31	14	31	4	3	14	31 (8.1)
		7	56	56	37	26	26	32	30	30	24	20	44	118 (11.5)
		14	35	27	24	29	26	30	6	5	21	49	129	204 (53.5)

Isolates susceptible to CLA on day 3 but resistant on day 14 were interpreted as exhibiting "inducible resistance" (6, 10, 11).

For each isolate, *erm*(41) sequevar and *rrl* and *rrs* gene sequences were analyzed and correlated with CLA and AMK profiles, as described previously (7, 11). Mutations of *rrl* and *rrs* sequences were compared with the published *M. abscessus* genome sequence (GenBank accession number NC_010397.1). *Erm*(41) sequence analysis was performed with ATCC 19977 (T28 sequevar, GenBank accession number FJ358483.1) and CR5701 (C28 sequevar, GenBank accession number HQ127366.1) as reference strains.

This study characterizes susceptibility profiles for the largest number of antibiotics to date for these clinical isolates, including resistance rates to macrolides and aminoglycosides (Table S1). AMK, CLA, and AZM were the three most effective agents against both *M. abscessus* subsp. *abscessus* (6/218, 2.8%; 14/218, 6.4%; 33/218, 15.1%, respectively) and *M. abscessus* subsp. *massiliense* isolates (7/163, 4.3%; 17/163, 10.4%; 21/163, 12.9%, respectively). Although the *M. abscessus* subsp. *abscessus* CLA resistance rate was higher on day 14 than on day 3 (84.9% versus 6.4%), the corresponding rate for subsp. *massiliense* barely changed (11.7% versus 10.4%). Meanwhile, similar overall trends were observed for AZM resistance (see Table S2 in the supplemental material). Notably, the acquired CLA resistance rate of 8.1% (31/381) was lower than those reported in other studies in China (33.95%) (12) and South Korea (15.84%) (13), higher than those reported in Brazil (5.55%) (14) and the United States (2.51%), and similar to the rate in France (9.09%) (15). These variations may be the results of differences in isolates or in patient treatment histories among the various studies.

Few studies reported from China have correlated antibiotic susceptibility with *M. abscessus* genotype for such a large number of samples. Here, based on subspecies and *erm*(41) sequevar characterizations of all 385 isolates, 218 were identified as *M. abscessus* subsp. *abscessus*, of which 185 possessed the *erm*(41) T28 sequevar and 33 possessed the *erm*(41) C28 sequevar. Another 163 isolates were classified as subsp. *massiliense*, and 4 other isolates belonged to *M. abscessus* subsp. *bolletii* and were excluded from further analysis due to their low representation. CLA MIC values are presented in Table 1 with regard to subspecies, *erm*(41) sequevar, and time of incubation. A total of 336 CLA-sensitive and 31 CLA-resistant subsp. *abscessus* isolates were

TABLE 2 Characterization of *M. abscessus* clinical isolates with phenotypic resistance to CLA and AMK

Genotype	No. of isolates with *rrl* mutation:				No. of isolates with *rrl* mutation/day 3 resistant (%)	No. of isolates with induced resistance (%)	No. of isolates with *rrs* mutation A1408G
	A2058C	A2058G	A2059C	Total			
erm(41) T28	4	2	1	7	7/31 (22.6)	171/174 (98.3)	3
erm(41) C28	2	1	0	3	3/31 (9.7)	0 (0)	2
M. massiliense	3	5	2	10	10/31 (32.3)	3/174 (1.7)	2

TABLE 3 Incubation time on MIC values and resistance rate of AMK antibiotic for *M. abscessus* subtype *erm*(41) sequevar

M. abscessus subtype	No. of isolates	No. of isolates at AMK MIC (μg/ml) of:											No. of resistant isolates (%)
		0.125	0.25	0.5	1	2	4	8	16	32	64	128	
T28	185	2	3	8	9	7	18	59	63	11	2	3	5 (2.7)
C28	33	0	1	1	5	1	3	5	14	2	1	0	1 (3.0)
massiliense	163	5	2	1	6	4	5	24	73	36	5	2	7 (4.3)
All subtypes	381	7	6	10	20	12	26	88	150	49	8	5	13 (3.4)

observed on day 3, whereas 204 CLA-resistant and 171 CLA-sensitive isolates were observed on day 14. Of CLA-sensitive isolates, most possessed the subsp. *massiliense* genotype ($n = 139$) or the *erm*(41) C28 ($n = 30$) sequevar. Notably, 2 CLA-sensitive isolates possessed *erm*(41) T28 sequevars, although the mechanism(s) underlying CLA sensitivity of these *erm*(41) T28 isolates are unknown.

The relationship between CLA susceptibility and *erm*(41) sequence results and *rrl* mutations with CLA susceptibility was also analyzed on day 3 of CLA exposure by assessing 194 CLA-sensitive (MIC, ≤2 μg/ml) and 92 CLA-intermediate (MIC, ≥4 μg/ml) subsp. *abscessus* isolates, all of which lacked *rrl* 2058/2059 mutations before CLA exposure. Of these isolates, 59 possessed the *erm*(41) C28 sequevar and 227 possessed the *erm*(41) T28 sequevar. As shown in Table 2, of 14 isolates exhibiting acquired resistance isolates (MIC, ≥8 μg/ml), 11 *erm*(41) T28 and 3 *erm*(41) C28 isolates were observed. Of the isolates with acquired resistance, 7 with the *erm*(41) T28 sequevar and all 3 with the *erm*(41) C28 sequevar also possessed an *rrl* 2058/2059 mutation. Of subsp. *massiliense* isolates, 142 were CLA-sensitive isolates, 4 were CLA-intermediate isolates, and 17 isolates exhibited acquired resistance. Of the latter, 10 possessed an *rrl* 2058/2059 mutation, and of these isolates, 5 harbored the A2058C mutation, 3 possessed the A2058G mutation, and 2 possessed the A2059C mutation. Because the remaining 7 resistant subsp. *massiliense* isolates exhibited no *rrl* 2058/2059 mutation, the underlying resistance mechanism was attributed to a change in 50S ribosomal subunit structure. Meanwhile, 3 *erm*(41) C28 isolates showed induced resistance to CLA, which was in agreement with results in previous studies showing that CLA resistance could be induced in isolates possessing the *erm*(41) C28 sequevar through *rrl* gene mutation after long-term exposure to high CLA concentrations.

Regarding AMK susceptibility, MIC values, subspecies, and *erm*(41) sequevar are shown in Table 3, with MIC values ranging from 0.125 to 128 μg/ml. AMK resistance rates of isolates with *erm*(41) T28 and *erm*(41) C28 sequevar profiles were 2.7% and 3.0%, respectively, with no significant difference between *erm*(41) sequevars. Meanwhile, 69.2% (9/13) of isolates with MICs of ≥64 μg/ml possessed an A1408G *rrs* mutation; 7 of these isolates resistant to both CLA and AMK harbored *rrl* and *rrs* mutations. Notably, all AMK-resistant isolates were associated with tobramycin MICs of ≥8 μg/ml. Therefore, *rrs* mutations conferred cross-resistance between AMK and tobramycin, as previously reported (15).

In conclusion, this study demonstrated an association between CLA drug resistance rate and *M. abscessus* genotype, with *erm*(41) sequevar and *rrl* and *rrs* genotypes being predictive of the resistance to CLA and AMK, respectively. This work should guide development of more effective treatment for *M. abscessus* infections.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01523-18.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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