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MECHANISMS OF RESISTANCE

Species Distribution and Macrolide Susceptibility of *Mycobacterium fortuitum* Complex Clinical Isolates

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ABSTRACT The understanding of species distribution and inducible macrolide resistance in the *Mycobacterium fortuitum* complex (MFC) is limited. Of 90 mostly respiratory MFC clinical isolates, half were *M. fortuitum*, followed by *M. peregrinum*, *M. porcinum*, *M. septicum*, and *M. conceptionense*. Most *M. fortuitum*, *M. porcinum*, and *M. septicum* isolates were inducibly resistant to clarithromycin, whereas two-thirds of the *M. peregrinum* isolates were clarithromycin susceptible. Clarithromycin-resistant *M. fortuitum* isolates exhibited common mutations of *erm*(39), potentially involved in clarithromycin resistance.

KEYWORDS *Mycobacterium fortuitum* complex, clarithromycin, drug resistance, nontuberculous mycobacteria

The *Mycobacterium fortuitum* complex (MFC), composed of several closely related species of nontuberculous mycobacteria (NTM), can cause human pulmonary and extrapulmonary infections. Based on identification of clinical isolates, the distribution of MFC species varies geographically. In Taiwan, Greece, and the United Kingdom, *M. fortuitum* was the second most frequently isolated NTM after members of the *M. avium* complex (23%, 21%, and 20%, respectively), whereas in South Korea and Japan, *M. fortuitum* accounted for only 8% and 2% of all clinical isolates, respectively (1). Currently, the MFC includes *M. fortuitum*, *M. peregrinum*, *M. porcinum*, *M. septicum*, *M. conceptionense*, *M. boenickei*, *M. houstonense*, *M. neworleansense*, *M. brisbanense*, *M. farcinogenes*, *M. senegalense*, and *M. setense* (2–4).

According to American Thoracic Society guidelines (5), 80% of *M. fortuitum* isolates are clarithromycin (CLR) susceptible. However, the guidelines recommend that macrolides be used with caution, due to the presence of the erythromycin-inducible methylase (*erm*) gene, which confers inducible resistance to macrolides in several NTM species (5). To date, only limited studies have reported that *M. fortuitum* clinical isolates harbor the *erm*(39) gene (6, 7). Moreover, there are no studies of the correlation between macrolide susceptibility and *erm*(39) sequevars of *M. fortuitum*. Additionally, it is not clear whether MFC species other than *M. fortuitum* possess inducible macrolide resistance.

The aims of this study were to elucidate the species distribution of MFC clinical isolates; to evaluate the isolates for the presence of macrolide resistance-related genes, such as *erm* and *rrl*; and to determine the association between macrolide susceptibility and *erm*(39) sequevars.

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TABLE 1 Reidentification and clarithromycin resistance of *M. fortuitum* complex clinical isolates

Characteristic	Value for the following species:				
	M. fortuitum	*M. peregrinum*[b]	*M. porcinum*	*M. septicum*	*M. conceptionense*
No. (%) of isolates identified by MLSA[a]	44 (49)	27 (30)	10 (11)	7 (8)	2 (2)
Patient data					
Median (interquartile range) age (yr)	62 (54–72)	58 (55–63)	65 (56–76)	49 (40–84)	43, 59
No. (%) of female patients	21 (48)	13 (48)	3 (30)	3 (43)	2 (100)
No. (%) of isolates from the following specimens:					
Respiratory specimen	42 (96)	27 (100)	10 (100)	7 (100)	2 (100)
Blood	1 (2)	0	0	0	0
Joint fluid	1 (2)	0	0	0	0
No. (%) of isolates with the following susceptibility to clarithromycin:					
Susceptible	2 (5)	18 (69)	0	0	2 (100)
Inducibly resistant	37 (84)	8 (31)	9 (90)	6 (86)	0
Resistant	5 (11)	0	1 (10)	1 (14)	0

[a]Abbreviation: MLSA, multilocus sequencing analysis.
[b]Drug susceptibility testing results for clarithromycin were not available for one *M. peregrinum* isolate.

A total of 90 MFC clinical isolates collected from August 2011 to December 2013 at Samsung Medical Center were included in our study, which was approved by the Institutional Review Board (IRB) of Samsung Medical Center (IRB no. 2008-09-016). Multilocus sequencing analysis was carried out as described previously (8). Drug susceptibility testing (DST) for CLR was performed using the broth microdilution method (9). The MIC of CLR was determined on days 3 and 14 after incubation, and MFC isolates were considered susceptible (MIC, $\leq$2 μg/ml at days 3 and day 14), resistant (MIC, $\geq$8 μg/ml at day 3), or inducibly resistant (susceptible at day 3 but resistant at day 14) to CLR (9). We designed species-specific PCR primers for sequencing of the entire *erm* gene: *erm*(39)fo F (5′-GAAATTGAGTTGAGCGTCCG-3′) and *erm*(39)fo R (5′-TCTACATCGCCTGGAC CATC-3′) for *erm*(39) in *M. fortuitum* and *erm*(39)po F (5′-CAGTGACCTACCTCCGCTTG-3′) and *erm*(39)po R (5′-CTACATCGCCTGGACCATCG-3′) for *erm*(39) in *M. porcinum*. The *erm*(39) sequences were trimmed using the CLUSTAL W program (10). Phylogenetic trees were obtained by the use of MEGA (version 6.0) software (11). To detect *rrl* mutations, PCR was performed as described previously (12).

Eighty-eight of the 90 MFC isolates were recovered from respiratory specimens, and 44 (49%) were reidentified as *M. fortuitum*, followed by 27 (30%) as *M. peregrinum*, 10 (11%) as *M. porcinum*, 7 (8%) as *M. septicum*, and 2 (2%) as *M. conceptionense* (Table 1). Among the 44 *M. fortuitum* isolates, 37 (84%) were inducibly resistant to CLR, 5 exhibited CLR resistance, and the remaining 2 were susceptible to CLR. Among the 27 *M. peregrinum* isolates, 18 (69%) were CLR susceptible and 8 (31%) were inducibly resistant to CLR, but DST results for CLR were not available for 1 isolate. Almost all *M. porcinum* and *M. septicum* isolates had inducible macrolide resistance, with only one of each species being resistant to CLR. Both *M. conceptionense* isolates were CLR susceptible. None of the 90 MFC isolates had *rrl* mutations, which can confer CLR resistance (12).

Whole *erm* gene sequencing was conducted for the 44 *M. fortuitum* and 10 *M. porcinum* clinical isolates, as these species are known for harboring *erm*(39) (13). Each of these sequences differed by at least 1 nucleotide from the *erm*(39) sequence of the *M. fortuitum* type strain DSM46621 (GenBank accession no. AY487229) (see Fig. S1 in the supplemental material). However, the sequences of 34 of the 44 isolates were identical to the *erm*(39) sequence of *M. fortuitum* strain CT6 (GenBank accession no. CP011269) (Fig. 1). Of the 45 single-nucleotide polymorphisms identified, 23 were synonymous (Fig. S1). The 16 amino acid substitutions are listed in Table 2. In all, nine *erm*(39) sequevars were identified and were numbered as sequevars 2 to 10. The sequevar of *M. fortuitum* type strain DSM46621 was designated sequevar 1. Thirty-seven of the 44 *M. fortuitum* isolates with inducible CLR resistance belonged to sequevars 2 to 5. Two CLR-

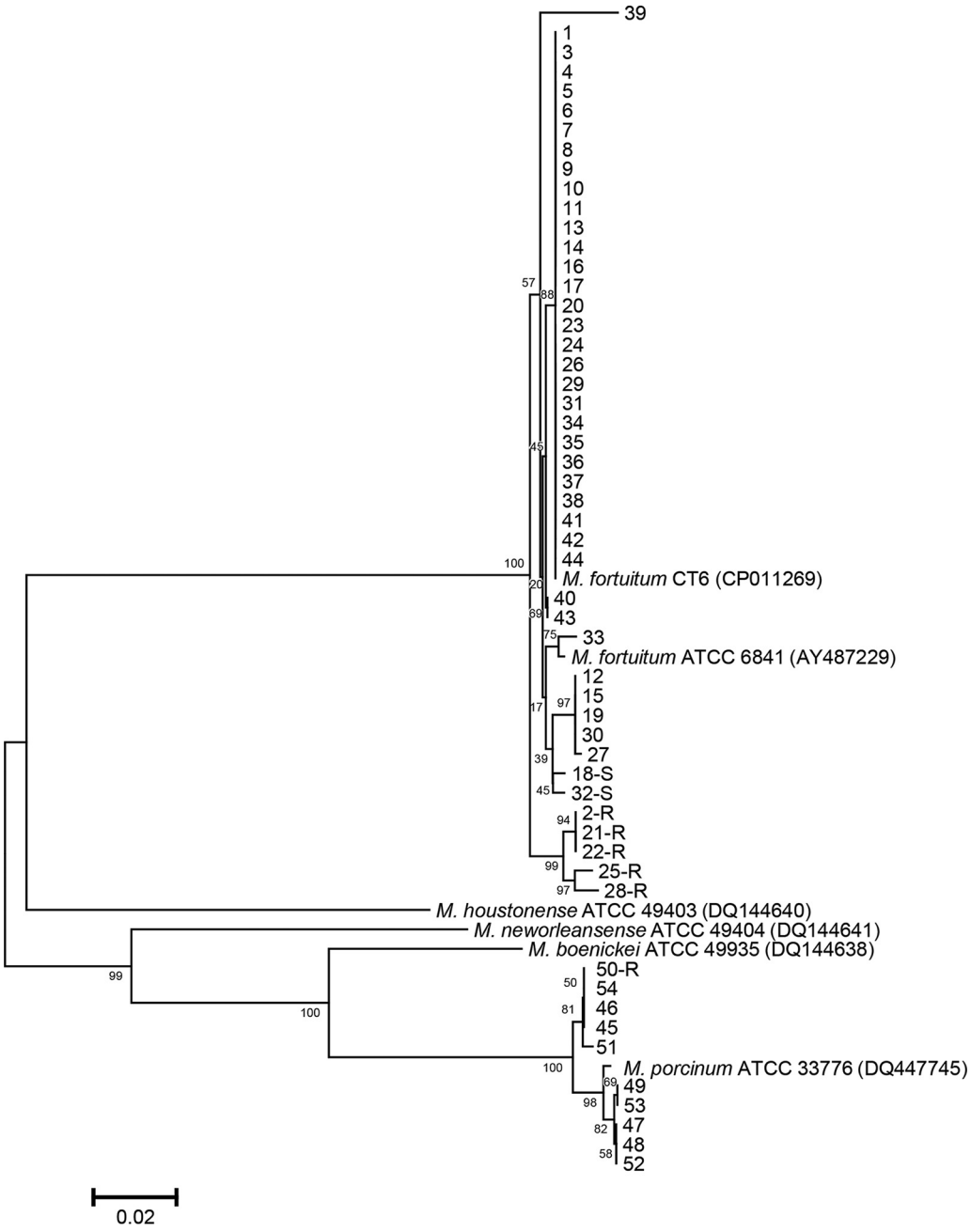

FIG 1 Phylogenetic tree for *M. fortuitum* complex (MFC) isolates derived using *erm*(39) sequences. Sequences were included for 44 *M. fortuitum* (isolate no. 1 to 44) and 10 *M. porcinum* (isolate no. 45 to 54) clinical isolates as well as other species belonging to the MFC. The clarithromycin-susceptible or -resistant isolates are indicated by an S or an R, respectively, after the isolate number. Sequences were compared with those of the type strains and other reference strains using the neighbor-joining method with Kimura's two-parameter distance correction model. Bootstrap analyses determined from 1,000 replicates are indicated at the nodes. Bar, 2% difference in nucleotide sequence. GenBank accession numbers are given in parentheses.

susceptible isolates had sequevars 6 and 7. Five CLR-resistant isolates had common mutations at nucleotide positions 76, 78, 661, 707, and 729 which were not shared by isolates of the other sequevars; these five isolates were divided among sequevars 8, 9, and 10. Therefore, these mutations found only in CLR-resistant isolates are potentially involved in the CLR resistance of *M. fortuitum*. Sequence variation in *erm* has also been noted in *M. abscessus*, with some mutations resulting in the loss of inducible macrolide resistance (14).

The *erm*(39) sequences of the 10 *M. porcinum* clinical isolates differed from the

TABLE 2 The 10 *erm*(39) gene sequevar types identified in *M. fortuitum*

Sequevar	No. of isolates	Susceptibility to CLR[a]	Nucleotide (amino acid)[b] at the following base pair position (corresponding amino acid amino acid position):															
			T11 (V4)	G19 (G7)	G64 (V22)	GTG 76-78 (V26)	G97 (A33)	G101 (G34)	A235 (I79)	G358 (A120)	T362 (I121)	C536 (A179)	G563 (R188)	G619 (A207)	G661 (A221)	C676 (P226)	G707 (R236)	T729 (D243)
1[c]	0		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
2	1	IR	●	●	●	●	●	●	●	T (S)	●	●	●	●	●	●	●	●
3[d]	34	IR	●	●	●	●	A (T)	●	G (V)	●	●	●	●	●	●	●	●	●
4[e]	1	IR	C (A)	●	A (I)	●	A (T)	●	G (V)	●	●	●	●	●	●	●	●	●
5	1	IR	●	●	●	●	A (T)	●	G (V)	●	●	●	●	●	●	A (T)	●	●
6	1	Susceptible	●	A (S)	●	●	A (T)	●	G (V)	●	●	●	●	●	●	●	●	●
7	1	Susceptible	C (A)	●	●	●	A (T)	C (A)	G (V)	●	●	●	●	●	●	●	●	●
8	3	Resistant	C (A)	●	●	ATC (I)	A (T)	●	G (V)	●	●	●	●	●	A (T)	●	A (Q)	A (E)
9	1	Resistant	C (A)	●	●	ATC (I)	A (T)	●	G (V)	●	●	A (D)	●	A (T)	A (T)	●	A (Q)	A (E)
10	1	Resistant	C (A)	●	●	ATC (I)	A (T)	●	G (V)	●	C (T)	●	A (H)	●	A (T)	●	A (Q)	A (E)

[a]Abbreviations: CLR, clarithromycin; IR, inducibly resistant.

[b]Nucleotides (amino acids) for sequevar type 1 are shown in the column subheads. ●, identical nucleotides. For altered nucleotides, the corresponding amino acid change is also indicated.

[c]Type strain DSM46621.

[d]Identical to the sequence of strain CT6.

[e]This isolate had the additional substitutions L99V, K156R, S158P, T174I, and P212S.

erm(39) sequence of the *M. porcinum* type strain ATCC 33776 (GenBank accession no. DQ447745) by 3 to 11 nucleotide mismatches (Fig. S2). Although we detected some shared mutations, there was no consistent association between a specific mutation and the macrolide resistance pattern.

The majority of *M. peregrinum* isolates were CLR susceptible in our study, but one-third had inducible macrolide resistance. *M. peregrinum* was originally reported to have no *erm* gene (13). However, a subsequent study revealed that 8 of 23 *M. peregrinum* clinical isolates had *erm* genes (7). Further studies are needed to resolve the role of the *erm* gene in this species.

The *M. septicum* type strain was reported to be susceptible to CLR using the Etest method with incubation for 3 days (15). However, we identified a putative *erm* gene in the *M. septicum* type strain (GenBank accession no. HG322951; the region from positions 2111635 to 2112375) with 86% identity to the sequences of *erm*(39) from *M. boenickei* and *M. houstonense* (GenBank accession no. DQ144638 and DQ144640, respectively). Additionally, six (86%) of our seven *M. septicum* clinical isolates had inducible resistance to CLR. These results provide further evidence that macrolide resistance in *M. septicum* can be inducible.

As in our results, *M. conceptionense* clinical isolates in two other studies were susceptible to CLR (16, 17). These findings suggest that *M. conceptionense* does not have a functional *erm* gene, but additional research is needed to determine whether this lack is a hallmark of the species.

In this study, we made several novel observations. First, 5% of the *M. fortuitum* clinical isolates were susceptible to CLR. Second, CLR-resistant *M. fortuitum* clinical isolates had several mutations in common in *erm*(39). Third, 30% of *M. peregrinum* isolates and most *M. septicum* isolates showed inducible macrolide resistance. To the best of our knowledge, this is the first report to investigate the association between *erm*(39) sequevars and resistance to CLR in *M. fortuitum*. This study highlights the importance of accurate species identification of MFC clinical isolates and of prolonged incubation during DST to screen for inducible macrolide resistance. Furthermore, as there can be variation in *erm* genes even within a species, such prolonged incubation may be of value even for NTM species not previously known to have inducible macrolide resistance.

Accession number(s). The *erm*(39) sequences were deposited in GenBank under accession numbers MK468741 to MK468794.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02331-18.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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