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Rapid Molecular Detection of Inducible Macrolide Resistance in *Mycobacterium chelonae* and *M. abscessus* Strains: a Replacement for 14-Day Susceptibility Testing?

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The *erm*(41) gene causes inducible macrolide resistance in *Mycobacterium abscessus* but not *Mycobacterium chelonae*. *erm*(41) sequencing of 285 *M. abscessus* and 45 *M. chelonae* isolates was compared to 14-day susceptibility; agreement percentages were 98.9% and 100%, respectively. Extended incubation may not be necessary for *M. chelonae*, and the *erm*(41) genotype is a useful adjunct for *M. abscessus*.

Mycobacterium abscessus is a rapidly growing mycobacterium (RGM) that is increasingly responsible for chronic pulmonary and cutaneous infections (1). Whole-genome sequence analysis supports the separation of this species into 3 taxa, including: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii* (2).

Infections due to these pathogens are often difficult to treat, in part because of acquired and/or intrinsic antimicrobial drug resistance. Clarithromycin and azithromycin remain the cornerstones of RGM therapy (3). Acquired macrolide resistance in the *M. abscessus* group, as well as the closely related *Mycobacterium chelonae*, has been linked to the point mutations A2058 and A2059 in the *rrl* gene encoding the peptidyltransferase domain of 23S rRNA. *rrl* gene mutants rarely emerge during therapy and are readily detected by routine 72-h susceptibility testing (4). An intrinsic macrolide resistance mechanism has also been described that involves an inducible erythromycin ribosomal methylase gene [*erm*(41)] (5). Previous work has demonstrated that (i) *M. abscessus* subsp. *abscessus* harbors an intact *erm*(41) gene but a C-to-T polymorphism at the 28th nucleotide position (T28) confers inducible clarithromycin resistance while C28 isolates remain susceptible (except for *rrl* mutants), (ii) *M. abscessus* subsp. *bolletii* strains had *erm*(41) sequences and resistance patterns similar to those of T28 *M. abscessus* subsp. *abscessus* isolates, and (iii) *M. abscessus* subsp. *massiliense* contained two deletions, rendering *erm*(41) nonfunctional and the organism macrolide susceptible (except for *rrl* mutants) (5–7). *M. chelonae* is not thought to contain *erm*(41), but this conclusion is based on relatively few genotyped isolates (5) and/or strains incubated in the presence of clarithromycin for >3 days (8).

To ensure detection of inducible macrolide resistance *in vitro*, the Clinical and Laboratory Standards Institute (CLSI) recommends that all RGM susceptibility testing for clarithromycin be determined after 14 days of incubation, unless resistance is recognized sooner (9). An alternative approach that would decrease the turnaround time to macrolide result reporting involves genomic interrogation of the *erm*(41) gene. (This work was presented in part at the 113th General Meeting of the American Society for Microbiology in Denver, CO, 18 to 21 May 2013.)

The purpose of this study was to verify that *erm*(41) sequencing correlates with extended microbroth susceptibility testing using a large number of prospectively collected *M. abscessus* group and *M. chelonae* isolates in a clinical setting. RGM specimens were submitted to the ARUP Reference Laboratories for identification and susceptibility testing between April 2011 and April 2013. Isolates were first identified to the *M. chelonae-abscessus* complex level by partial 16S rRNA gene sequencing (10). Differentiation between *M. chelonae* and the *M. abscessus* group was accomplished using real-time internal transcribed spacer PCR analysis as previously described (11, 12). Macrolide susceptibility testing was performed per the 2011 CLSI guideline using RGM Sensititre MIC plates (Trek Diagnostic Systems, Cleveland, OH). Clarithromycin results were read after 3, 5, 7, 12, and 14 days of incubation or until the time resistance was first detected.

During the 2-year study period, 427 *M. chelonae* and 1,025 *M. abscessus* group isolates were identified as a part of routine clinical care, but patient histories were not available for review. Phenotypic clarithromycin susceptibilities are displayed by day of incubation in Fig. 1. All *M. chelonae* isolates were clarithromycin susceptible after 3 days of incubation, which suggests an absence of *rrl* mutants in this sample. The majority of *M. chelonae* strains (426/427, 99.8%) were fully susceptible at day 14, in agreement with previous reports that clarithromycin MICs did not increase when the incubation times were extended (8). In contrast, a small proportion of *M. abscessus* group isolates (28/1025, 2.7%) appeared resistant after 3 days and more than half (637/1025, 62.1%) were ultimately reported as resistant after a week or more of incubation (i.e., inducibly resistant).

A subset of unique *M. chelonae* and *M. abscessus* group isolates from individual patients, representing all of the observed clarithromycin susceptibility profiles, were selected for *erm*(41) se-

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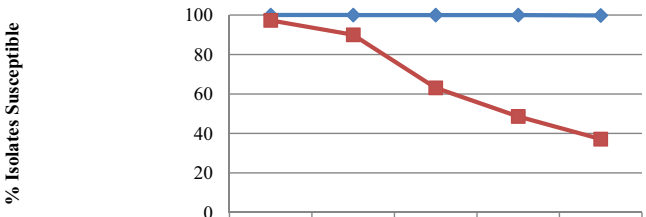

FIG 1 Fourteen-day clarithromycin susceptibility test results for all isolates over a 2-year period. One *M. chelonae* and 3 *M. abscessus* group isolates had intermediate clarithromycin susceptibility (MIC, 4 μg/ml) detected at day 14 on repeated testing. These organisms are classified here as nonsusceptible.

quencing. Briefly, isolates were retrieved from liquid nitrogen storage and checked for purity. The *erm*(41) PCR mixture contained the following: 0.5 μM of primers (5), 1× GoTaq colorless master mix (Promega, Madison, WI) with a final MgCl concentration of 1.5 mM, 5 μl template DNA, and 200 μM of each deoxynucleoside trisphosphate (dNTP). Step-down PCR was performed on an ABI 9700 thermal cycler. Cycling conditions included a denaturation hold at 94°C for 10 min, 20 cycles at 94°C for 10 s, 65°C (with a 1° drop per cycle) for 30 s, 72°C for 60 s, 15 cycles of 94°C for 10 s, 35°C for 20 s, 72°C for 60 s, and a final primer extension at 72°C for 2 min. Amplicons were detected on a 2% agarose gel and purified using Agencourt AMPure magnetic beads (Beckman Coulter, Brea, CA). Sanger sequencing using BigDye Terminator chemistry was then performed on the ABI Prism 3730 DNA analyzer per the manufacturer's instructions. Sequences obtained for *erm*(41) were assembled in VectorNTI Advance 11.0 Contig Express (Life Technologies, Grand Island, NY), aligned in MEGA 5.2 (13), and compared to the NCBI Reference Sequence Database using BLAST analysis. The *erm*(41) sequences and predicted macrolide susceptibility were interpreted per Nash et al. (5).

In all, 330 isolates (*M. chelonae* [$n = 45$] and *M. abscessus* group [$n = 285$]) that were macrolide susceptible after 72 h of incubation had *erm*(41) sequencing performed (Table 1). None of the 45 *M. chelonae* isolates analyzed, including a single strain with intermediate susceptibility at day 14, contained the *erm*(41) gene. Interestingly, the *erm*(41) primers did amplify highly similar sequences for some isolates (21/45, 46.7%), but none matched references deposited in GenBank (data not shown). These observations, taken together with the phenotypic susceptibility results presented in Fig. 1, suggest that holding *M. chelonae* isolates for 14 days to exclude inducible clarithromycin resistance may not be necessary. A total of 285 *M. abscessus* group isolates were also sequenced. Most genotyped isolates were from respiratory (62.1%) sources, followed by skin/soft tissue (18.9%) or blood (5.6%). Initially, 5 isolates with susceptible *erm*(41) genotypes (C28 sequevars) were read as intermediately susceptible to clarithromycin on day 14. Sequence alignments revealed no novel *erm*(41) polymorphisms in these isolates. Repeat susceptibility testing reclassified 2 of the 5 as fully susceptible (MIC, ≤2 μg/ml) and 3 remained intermediate on day 14 only (MIC, 4 μg/ml). Following this discrepancy resolution testing, overall agreement between the *M. abscessus* group macrolide susceptibility genotype and phenotype was 98.9% (282/285). If the intermediately susceptible isolates of uncertain clinical significance were excluded, there was 100% concordance.

Subspecies-level identification of the *M. abscessus* group has been recommended based on the observation that patients with *M. abscessus* subsp. *abscessus* lung infections have poorer clinical and microbiologic responses to antimicrobial therapy than do those with *M. abscessus* subsp. *massiliense* infections (14). Outcome differences are likely related to the inducible macrolide resistance more commonly associated with *M. abscessus* subsp. *abscessus* strains. We did not attempt to differentiate members of the *M. abscessus* group to also determine if subspecies identification matched the expected *erm*(41) sequence. Procedures for identification to the species level are not trivial and require multilocus sequence analysis (15–17), a dedicated gel-based multiplex PCR targeting regions of genetic difference (18), or possibly manual analysis of individual peak differences contained within matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) spectra (19). Multilocus sequence typing and principal component cluster-based analysis of protein spectra are beyond the scope of most clinical laboratories. Gel-based PCR assays are more labor-intensive than automated real-time systems but do offer a more practical solution for this application. However, gene transfer across members of the *M. abscessus* group limits the inference of subspecies-specific susceptibility patterns (15). For example, 2 *M. abscessus* subsp. *massiliense* strains with full-length *erm*(41) gene sequences and inducible macrolide resistance were recently reported (18). Thus, predicting macrolide susceptibility based on subspecies in these cases would have been inaccurate. While identification to the species level may be useful for epidemiologic purposes, perhaps the most important information for immediate patient care is whether or not an *M. abscessus* group isolate harbors a wild-type *erm*(41) gene or *rrl* mutations.

In line with previous reports (5–7), we observed strong overall agreement (98.9%) between the *erm*(41) genotype and 14-day clarithromycin susceptibility for a large number of *M. abscessus* group isolates that appeared susceptible after 3 days of initial incubation. Discrepant results were derived from 3 *M. abscessus* isolates that had reproducibly intermediate clarithromycin results on day 14 but lacked a functional *erm*(41) gene. These would be re-

TABLE 1 Correlation between *erm*(41) genotype and macrolide phenotypic susceptibility for selected *M. chelonae-abscessus* complex isolates

ITS[a] identification (*n*)	14-day clarithromycin susceptibilities[b] of isolates (no. [%])			*erm*(41) genotype
	Susceptible (MIC ≤ 2 μg/ml)	Intermediate (MIC 4 μg/ml)	Resistant (MIC ≥ 8 μg/ml)	
M. chelonae (45)	44 (97.8)	1 (2.2)	0	Not present
M. abscessus group (285)	74 (26)	2 (0.7)	0	C28 sequevar
	0	0	94 (33.0)	T28 sequevar
	114 (40)	1 (0.3)	0	Deletions[c]

[a] ITS, internal transcribed spacer.
[b] All sequenced isolates were clarithromycin susceptible after 72 h of incubation in an attempt to eliminate organisms with acquired macrolide resistance due to *rrl* mutation.
[c] Observed deletions, which always occurred together, included deletions at nucleotides 64 and 65 and a deletion of 274 bp starting from nucleotide 159 (using the GTG start codon as nucleotide 1).

ported as susceptible based on *erm*(41) genotype (i.e., C28 sequevar). Whether this difference would adversely affect patient treatment outcomes is not known. In addition, *M. chelonae* does not appear to harbor an inducible macrolide resistance mechanism.

In conclusion, determining *M. chelonae* and *M. abscessus* group macrolide susceptibility after 3 to 5 days of incubation combined with *erm*(41) sequence analysis is a rapid, highly accurate, and logistically feasible approach for the clinical laboratory. The elimination of 14-day clarithromycin susceptibility testing for *M. chelonae* and perhaps also *M. abscessus* group isolates could streamline clinical laboratory workflow, but additional studies are required to assess the cost-effectiveness and clinical impact of rapid molecular-based approaches. For now, *erm*(41) sequence analysis for the *M. abscessus* group may be useful as an adjunct to extended susceptibility testing.

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