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GenoType NTM-DR for Identifying *Mycobacterium abscessus* Subspecies and Determining Molecular Resistance

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We studied the performance of a new line probe assay for identifying the subspecies and determining the macrolide and aminoglycoside resistance levels of 50 *Mycobacterium abscessus* isolates. Agreement of GenoType NTM-DR results with sequencing and phenotypic resistance results was 92% for subspecies identification and 98% for determining molecular and phenotypic resistance.

The *Mycobacterium abscessus* complex is divided into three subspecies: *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii*. Their differentiation is of clinical interest, because subspecies differ in antibiotic resistance and treatment response in *M. abscessus* lung disease (1, 2). Identification of the members of *M. abscessus* relies on sequencing of multiple genes (3, 4). Inducible macrolide resistance in *M. abscessus* is conferred by the presence of the inducible methylase Erm(41) (5, 6), whereas high-level clarithromycin resistance is attributed to mutations at position 2058 or 2059 in the peptidyltransferase-binding region of the 23S rRNA gene (*rrl*) (7). A single point mutation at position 1408 in *rrs* of the 16S rRNA gene is responsible for the high level of resistance against aminoglycosides, another category of first-line antibiotics (8).

GenoType NTM-DR (Hain Lifescience, Nehren, Germany) is a new line probe assay that enables *M. abscessus* subspecies identification and the simultaneous determination of antibiotic resistance to macrolides and aminoglycosides of mutations at position 28 in *erm*(41) (5, 6), position 2058/2059 in *rrl*, and position 1408 in *rrs*. We studied the ability of this assay to characterize 50 *M. abscessus* isolates (28 *M. abscessus* subsp. *abscessus* isolates, 19 *M. abscessus* subsp. *massiliense* isolates, and 3 *M. abscessus* subsp. *bolletii* isolates). Furthermore, 4 *Mycobacterium chelonae* isolates were analyzed. *M. chelonae* is closely related to *M. abscessus*. The two species share the same biochemical features, have highly similar 16S rRNA sequences, and are frequently summarized as the *M. chelonae/abscessus* complex (9). Results of the GenoType NTM-DR assay were compared with the results of sequencing the *hsp65*, *erm*(41), *rrl*, *rrs*, and 16S rRNA genes, which were obtained previously (10) or sequenced within this study as described in reference 10. Additionally, molecular resistance results of all *M. abscessus* isolates were compared with results of phenotypic resistance to clarithromycin and amikacin published by Rueger et al. (10), who used the broth microdilution method in RAPMYCO Sensititre 96-well plates. According to the CLSI breakpoints, high-level clarithromycin resistance was defined as an MIC of ≥8 μg/ml on day 5, and inducible resistance was defined as an increase of the clarithromycin MIC from ≤2 μg/ml on day 5 to ≥8 μg/ml on day 14. Aminoglycoside resistance was defined as an amikacin MIC of ≥64 μg/ml.

Isolates were grown in mycobacterial growth indicator tube (MGIT) liquid medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 37°C. GenoType NTM-DR was performed according to the manufacturer's recommendations.

Of the 50 *M. abscessus* isolates studied, 46 (92%) exhibited subspecies identification results concordant with the results obtained by DNA sequencing (Table 1). The banding patterns of three isolates were not attributed to a subspecies, namely, of one *M. abscessus* subsp. *abscessus* and two *M. abscessus* subsp. *massiliense* isolates. DNA sequencing of the unidentified *M. abscessus* subsp. *massiliense* isolates did not reveal deletion at position 64/65 within *erm*(41); such a deletion is a typical feature of *M. abscessus* subsp. *massiliense* (5, 6). The unidentified *M. abscessus* subsp. *abscessus* isolate did not exhibit sequence abnormalities within the 16S rRNA, *erm*(41), or *hsp65* gene amplicon. One other isolate, identified as *M. abscessus* subsp. *abscessus* by gene sequencing, was identified as *M. abscessus* subsp. *bolletii* by the GenoType NTM-DR.

GenoType NTM-DR results matched 100% (50/50) of the *erm*(41) and *rrs* sequencing results and 98% (49/50) of the *rrl* sequencing results (Table 1). Overall, 9 isolates exhibited mutations within *rrl*, and 7 isolates exhibited mutations within *rrs*. One *M. abscessus* subsp. *abscessus* isolate exhibited a mutation in *rrl* that revealed neither a mutation band nor a wild-type band, a finding indicating the existence of a resistance mechanism not detected by the line probe assay.

The four *M. chelonae* isolates were correctly identified by the GenoType NTM-DR, and resistance results correlated with the sequencing results of *rrs* and *rrl*, which all exhibited a wild-type sequence (data not shown). *erm*(41) is absent in *M. chelonae* (5).

Phenotypic high-level clarithromycin resistance testing of the 50 *M. abscessus* isolates showed congruence with the molecular methods (Table 1). The 9 isolates, which had been tested resistant

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TABLE 1 Sequencing and Phenotypic Resistance Results of 50 *Mycobacterium abscessus* Isolates[a]

No.	*Mycobacterium abscessus* subspecies (sequencing)	Position 28 in *erm*(41)	Clarithromycin MIC (μg/ml) on day 5	Clarithromycin MIC (μg/ml) on day 14	*rrl* positions 2058/2059	Amikacin MIC (μg/ml) on day 5	*rrs* position 1408
1	*Abscessus*	T (R)	0.5 (S)	>16 (R)	AA	4 (S)	A
2	*Abscessus*	T (R)	2 (S)	>16 (R)	AA	4 (S)	A
3	*Abscessus*	C (S)	0.5 (S)	2 (S)	AA	16 (S)	A
4	*Abscessus*	C (S)	0.25 (S)	0.25 (S)	AA	8 (S)	A
5	*Abscessus*	C (S)	>16 (R)	>16 (R)	CA (R)	>64 (R)	G (R)
6	***Massiliense***	T (S)	0.06 (S)	0.5 (S)	AA	8 (S)	A
7	***Massiliense***	T (S)	>16 (R)	>16 (R)	AG (R)	>64 (R)	G (R)
8	*Massiliense*	T (S)	0.25 (S)	0.5 (S)	AA	4 (S)	A
9	*Massiliense*	T (S)	0.25 (S)	1 (S)	AA	>64 (R)	G (R)
10	*Abscessus*	T (R)	0.5 (S)	>16 (R)	AA	4 (S)	A
11	*Abscessus*	T (R)	0.25 (S)	>16 (R)	AA	4 (S)	A
12	*Massiliense*	T (S)	>16 (R)	>16 (R)	CA (R)	>64 (R)	G (R)
13	*Massiliense*	T (S)	>16 (R)	>16 (R)	CA (R)	>64 (R)	G (R)
14	*Massiliense*	T (S)	0.5 (S)	1 (S)	AA	16 (S)	A
15	*Massiliense*	T (S)	0.5 (S)	1 (S)	AA	16 (S)	A
16	*Massiliense*	T (S)	0.25 (S)	0.5 (S)	AA	16 (S)	A
17	*Massiliense*	T (S)	0.25 (S)	0.5 (S)	AA	16 (S)	A
18	*Abscessus*	C (S)	0.06 (S)	0.12 (S)	AA	8 (S)	A
19	*Massiliense*	T (S)	0.5 (S)	0.5 (S)	AA	8 (S)	A
20	***Abscessus***	T (R)	2 (S)	>16 (R)	AA	16 (S)	A
21	*Abscessus*	T (R)	0.5 (S)	>16 (R)	AA	16 (S)	A
22	*Abscessus*	T (R)	>16 (R)	>16 (R)	**TA (R)**	32 (I)	A
23	*Abscessus*	T (R)	4 (I)	>16 (R)	AA	16 (S)	A
24	*Massiliense*	T (S)	0.25 (S)	1 (S)	AA	8 (S)	A
25	*Massiliense*	T (S)	0.06 (S)	0.5 (S)	AA	8 (S)	A
26	*Massiliense*	T (S)	0.25 (S)	2 (S)	AA	8 (S)	A
27	*Massiliense*	T (S)	0.25 (S)	0.25 (S)	AA	8 (S)	A
28	*Massiliense*	T (S)	1 (S)	2 (S)	AA	8 (S)	A
29	*Abscessus*	T (S)	1 (S)	>16 (R)	AA	32 (I)	A
30	*Abscessus*	T (R)	0.5 (S)	>16 (R)	AA	16 (S)	A
31	*Abscessus*	T (R)	0.25 (S)	>16 (R)	AA	8 (S)	A
32	*Abscessus*	T (R)	1 (S)	>16 (R)	AA	**>64 (R)**	A
33	*Abscessus*	T (R)	0.25 (S)	16 (R)	AA	4 (S)	A
34	*Abscessus*	T (R)	1 (S)	>16 (R)	AA	4 (S)	A
35	*Abscessus*	T (R)	0.25 (S)	16 (R)	AA	8 (S)	A
36	*Massiliense*	T (S)	0.25 (S)	1 (S)	AA	8 (S)	A
37	*Abscessus*	T (R)	1 (S)	>16 (R)	AA	32 (I)	A
38	*Abscessus*	C (S)	0.12 (S)	0.25 (S)	AA	16 (S)	A
39	*Abscessus*	C (S)	0.25 (S)	1 (S)	AA	8 (S)	A
40	*Massiliense*	T (S)	0.12 (S)	0.25 (S)	AA	16 (S)	A
41	*Abscessus*	T (R)	0.5 (S)	>16 (R)	AA	8 (S)	A
42	*Massiliense*	T (S)	>16 (R)	>16 (R)	CA (R)	8 (S)	A
43	*Abscessus*	T (R)	0.5 (S)	>16 (R)	AA	32 (I)	A
44	*Abscessus*	T (R)	2 (S)	>16 (R)	AA	32 (I)	A
45	*Bolletii*	T (R)	8 (R)	>16 (R)	AA	16 (S)	A
46	*Bolletii*	T (R)	4 (I)	>16 (R)	AA	16 (S)	A
47	***Abscessus***	C (S)	>16 (R)	>16 (R)	AG (R)	>64 (R)	G (R)
48	*Abscessus*	T (R)	>16 (R)	>16 (R)	GA (R)	16 (S)	A
49	*Bolletii*	T (R)	16 (R)	>16 (R)	AA	16 (S)	A
50	*Abscessus*	C (S)	>16 (R)	>16 (R)	AG (R)	>64 (R)	G (R)

[a] Disagreements with GenoType NTM-DR results are marked in bold. One *M. abscessus* subsp. *abscessus* isolate was identified as *M. abscessus* subsp. *bolletii* by the GenoType NTM-DR. The three other disagreeing isolates did not yield species-specific band patterns in the GenoType NTM-DR. R, resistant; S, susceptible; I, intermediate; C, cytosine; T, thymine; A, adenine; G, guanine.

by *rrl* sequencing, exhibited phenotypic high-level resistance to clarithromycin (MICs > 16 μg/ml) after 5 days.

Phenotypic inducible clarithromycin resistance was detected by increases in the clarithromycin MICs from day 5 to day 14 in 18 *M. abscessus* subsp. *abscessus* isolates. These results are in line with the results of molecular testing by the GenoType NTM-DR and sequencing for these isolates. Two more *M. abscessus* subsp. *abscessus* isolates exhibited inducible and high-level clarithromycin

resistance. A clarithromycin MIC of >16 μg/ml on day 5, explained by high-level clarithromycin resistance, masked the inducible resistance in these isolates. No inducible phenotypic macrolide resistance was found in *M. abscessus* subsp. *massiliense*, which is explained by the lack of a functional *erm*(41) gene (11). A thymine at position 28 is not accompanied by inducible clarithromycin resistance in this subspecies. Three *M. abscessus* subsp. *bolletii* isolates exhibited induced clarithromycin resistance but not high-level macrolide resistance, as determined by sequencing and the GenoType NTM-DR. These isolates showed MICs of 4–16 μg/μl on day 5 and >16 μg/ml on day 14, indicating a faster induction of resistance. Of the 50 *M. abscessus* isolates, 8 isolates exhibited phenotypic aminoglycoside resistance (3 *M. abscessus* subsp. *abscessus* and 5 *M. abscessus* subsp. *massiliense* isolates) with an amikacin MIC of >64 μg/ml. Seven of these isolates had been identified to be aminoglycoside resistant by the GenoType NTM-DR and sequencing of *rrs* position 1408. One isolate with an amikacin MIC of >64 μg/ml exhibited mutation at neither position 1408 nor positions 1406, 1409, or 1491 of *rrs*, which have been reported to cause aminoglycoside resistance in *M. abscessus* species (12). To summarize, the GenoType NTM-DR showed 98% correlation with molecular and phenotypic results for determining clarithromycin and aminoglycoside resistance. Phenotypic amikacin resistance of one *M. abscessus* subsp. *abscessus* isolate was not identified by the GenoType NTM-DR.

To the best of our knowledge, this is the first study to have evaluated a line probe assay for the simultaneous determination of *M. abscessus* subspecies and resistance to clarithromycin and aminoglycosides. The GenoType NTM-DR is a valuable test for characterizing most clinical *M. abscessus* isolates.

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We declare no conflicts of interest.

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