Evaluation of the GenoType® NTM DR for Subspecies Identification and Determination of Drug Resistance in Clinical *M. abscessus* Isolates

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**Abstract**

**Introduction:** A new line probe assay, the GenoType® NTM DR, has been developed for subspecies identification and detection of resistance to macrolides and aminoglycosides in clinical *Mycobacterium abscessus* isolates. We studied the performance of the test compared to DNA sequencing and phenotypic drug susceptibility testing (pDST).

**Methods:** 48 clinical *M. abscessus* isolates collected between 2015 and 2016 were identified to the subspecies level and analysed for *erm* (41) genotype, rrl and rrs gene mutations by Sanger sequencing. Broth micro dilution was performed for pDST of Clarithromycin and Amikacin. The results were compared to those of the GenoType® NTM DR assay. Discordant results were further analysed by repeat pDST and whole genome sequencing (WGS).

**Results:** 35 isolates were identified as *M. abscessus* subsp. *abscessus*, 6 as *M. abscessus* subsp. *bolletii*, and 7 as *M. abscessus* subsp. *massiliense* based on *rpoB* sequences. Concordance of GenoType® NTM DR results with Sanger sequencing was 92% for subspecies identification and 100% for *erm* (41), rrl, and rrs genotypes, respectively. GenoType® NTM DR and pDST results matched in 98% for Clarithromycin resistance and in 96% for Amikacin resistance when repeat pDST results were taken into account.

**Conclusion:** The new GenoType® NTM DR assay is a valuable test for subspecies identification of *M. abscessus* isolates and detection of defined mutations conferring resistance to Amikacin and Clarithromycin. Discrepancies between the line probe assay and pDST mainly relate to variations in phenotypic test results.

**Keywords:** *M. abscessus*, GenoType® NTM DR; Subspecies identification; Resistance testing; Whole genome sequencing

**Abbreviations:** A: Adenine; Absc: *M. abscessus* subsp. *abscessus*; Boll: *M. abscessus* subsp. *bolletii*; C: Cytosine; G: Guanine; I: Intermediate Resistance; INT: Interpretation; IR: Inducible Resistance; Massil: *M. abscessus* subsp. *massiliense*; MIC: Minimal Inhibitory Concentration; MUT: Mutation; R: Resistant; Subsp.: Subspecies; S: Susceptible; T: Thymine; WT: Wild Type.

**Introduction**

*Mycobacterium abscessus* is an emerging pathogen belonging to the rapidly growing mycobacteria. The spectrum of disease includes respiratory infections, especially in patients with chronic lung disease, as well as skin, soft tissue and bone infections, and rarely disseminated disease in severely immunosuppressed patients [1]. The *M. abscessus* complex has recently been divided into three subspecies, *M. abscessus* subsp. *abscessus* (or *M. abscessus sensu strictu*), *M. abscessus* subsp. *massiliense* and *M. abscessus* subsp. *bolletii* [2-4]. Macrolides and Amikacin are key drugs in the treatment of *M. abscessus* disease [1,5]. However, macrolide susceptibility varies by subspecies and may explain the association between treatment outcomes and subspecies [6-8]. The primary innate mechanism of macrolide resistance in *M. abscessus* is the inducible expression of an erythromycin ribosomal methylase, Erm(41). Erm(41) methylates the adenine at position 2058 of the 23S ribosomal RNA leading to reduced binding of macrolides to their target site in the 50S ribosomal subunit [9]. *M. abscessus* subsp. *bolletii* and *M. abscessus* subsp. *massiliense* harbour intact *erm*(41) genes. However, a T/C polymorphism at position 28 in *M. abscessus* subsp. *massiliense* results in inducible resistance (T28) or phenotypic susceptibility (C28), respectively [10]. *M. abscessus* subsp. *massiliense* has a dysfunctional *erm*(41) gene due to a 2 bp deletion of nucleotides 64–65 and a 274 bp deletion of nucleotides 159–432 leading to a macrolide susceptible phenotype [9]. In addition, acquired high-level resistance to macrolides is due to point mutations at positions 2058 and 2059 in the 23S rRNA (rrl) gene [11]. Acquired high-level resistance to aminoglycosides is conferred by point mutations at...
position 1408 of the 16S rRNA (rrs) gene and can occur in all three subspecies [12]. Today, subspecies identification and detection of genetic resistance markers for *M. abscessus* are based on sequencing of these specific target genes [13]. Recently a commercial line probe assay, the GenoType® NTM DR (HAIN Lifescience, Nehren, Germany) has been developed. It provides subspecies identification based on the ermA(41) gene variants, detection of point mutations in rrl and rrs, as well as determination of the T/C 28 polymorphism of ermA(41). This study aimed to compare the GenoType® NTM DR assay with DNA Sanger sequencing and phenotypic resistance results in isolates obtained from patient samples, using whole genome sequencing (WGS) to resolve discrepancies.

Materials and Methods

48 non-duplicate *M. abscessus* isolates obtained from the respiratory tract (sputum, bronchial secretion, bronchoalveolar lavage, blood, n=34), skin (n=1) or of unknown origin (n=13) were included in the study. Strains were identified at the German National Mycobacterium Reference Laboratory from July 2015 to January 2016 using the GenoType® CM assay (HAIN Lifescience, Nehren, Germany), and Sanger sequencing of the 16S and rpoB genes using an ABI Prism 3100 capillary sequencer with BigDye Terminator v.1.1 chemistry (Applied Biosystems, Foster City, CA) [14,15]. For subspecies identification, rpoB sequences were compared with GenBank accession numbers AY262741.1, AY593981.2 and AY859692.1 as previously [12]. Today, subspecies results were included in the analysis for a tree-based phylogenetic inference using BioNumerics v7.5 (Applied Maths, Belgium). Additional reference strains for isolates with discordant subspecies results were included in the analysis for a tree-based phylogenetic classification (*M. abscessus* subsp. *abscessus* ATCC 19977T, *M. abscessus* subsp. boletii CCUG 50184T and *M. massiliense* CCUG 48989T). Sequence data were submitted to the ENA sequence read archive (accession number ERP021910). Ethical approval was not sought as a new test was evaluated against standard assays using leftover samples already collected for clinical and laboratory assessment. No additional information of patients was sought and no patient contact was required. The results did not influence clinical decision-making. Concordance of results was calculated comparing proportions across different assays.

**Results**

Subspecies identification and initial drug susceptibility data of the 48 tested *M. abscessus* clinical isolates are summarised in Table 1. Altogether, 11 discordant results were initially found comparing GenoType® NTM DR to Sanger sequencing and pDST (isolate 5, 20, 21, 30–32, 34, 38–41).

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Subspecies identification by Sanger sequencing revealed 35 *M. abscessus* subsp. *abscessus*, six *M. abscessus* subsp. *boletii* and seven *M. abscessus* subsp. *massiliense* isolates. There was concordance of 92% (44/48) between Sanger sequencing and GenoType® NTM DR results. The four discordant isolates (isolates 38-41) were identified as *M. abscessus* subsp. *abscessus* by rpoB sequencing and as *M. abscessus* subsp. *massiliense* using the GenoType® NTM DR assay. WGS was performed on these isolates, confirming GenoType® NTM DR subspecies identification for all four isolates.

Sanger sequencing and GenoType® NTM DR showed 100% (48/48) agreement for the erm(41) T/C28 polymorphism and 100% (48/48) for analysis of rrl positions 2058 and 2059. For three isolates (isolates 29-31) Sanger sequencing revealed a mutation at position 2057, replacing adenine with guanine in two isolates and adenine with cytosine in one isolate. The pDST results for Clarithromycin were in accordance with the results predicted by GenoType® NTM DR (susceptible in isolate 29, and inducibly resistant in isolates 30 and 31).

GenoType® NTM DR results for Clarithromycin were concordant with initial pDST results in 94% (45/48). Repeat pDST of two *M. abscessus* subsp. *abscessus* isolates (isolates 20 and 21), previously tested susceptible, showed inducible resistance in concordance with both the GenoType® NTM DR and the sequence of erm(41). Repeat pDST of the third isolate (*M. abscessus* subsp. *boletii*, isolate 34), initially tested intermediate (MIC 4 μg/ml at day 14), showed susceptibility (MIC 2 μg/ml at day 14). GenoType® NTM DR and Sanger sequence data for position 28 of the erm(41) gene (thymine) indicated inducible resistance for this isolate. WGS of isolate 34 revealed several amino acid exchanges within the erm(41) gene. No frameshifts or deletions were observed.

Three of the 48 isolates showed discordant results for Amikacin between GenoType® NTM DR and initial pDST. Repeat pDST resolved one of the discrepancies (isolate 31, initially falsely susceptible in pDST). Two isolates (isolate 30 and 32) were repeatedly tested resistant to Amikacin (MIC>64 μg/ml), with pDST results 1408 being wildtype in both GenoType® NTM DR and Sanger sequencing. WGS showed no mutation across the whole rrs gene for isolate 34, and two mutations at rrs positions 1305 (adenine to cytosine) and 1306 (thymine to adenine) in isolate 32.

**Discussion**

In this study, results of the GenoType® NTM DR assay and Sanger DNA sequencing were concordant in 92% for subspecies differentiation, in 100% for the erm(41) T/C28 nucleotide polymorphism, in 100% for rrl positions 2058 and 2059, and in 100% for pDST results for Clarithromycin or Amikacin resistance. GenoType® NTM DR showed concordance with pDST in 94% for Clarithromycin resistance and in 94% for Amikacin resistance, improving to 98% and 96%, respectively, after repeat pDST. Similar results have recently been published by Kehrmann et al. [18] and Mougari et al. [19], however discordance of subspecies identification or resistance results did not take into account WGS data.

Four isolates (isolates 38-41) were identified as *M. abscessus* subsp. *abscessus* by sequencing of the rpoB gene alone, but as *M. abscessus* subsp. *massiliense* by GenoType® NTM DR, differentiating the subspecies on the basis of the erm(41) gene variants, and WGS. All four isolates displayed Clarithromycin susceptibility patterns compatible with *M. abscessus* subsp. *massiliense* (susceptible on days 3 and 14) and an erm(41) sequence showing the characteristic deletions of nucleotides 64–65 and 159–432. This has been described by others [20] and reflects the uncertainty of subspecies differentiation for *M. abscessus* when using a single gene like rpoB. Here, WGS can be of advantage as it allows more accurate assessment of an individual isolate in comparison to reference genomes of all three *M. abscessus* subspecies. The importance of subspecies identification for clinical practice is still a matter of debate. Some studies investigating the
association between subspecies identification and treatment outcomes in patients with pulmonary disease found better results for *M. abscessus* subsp. *massiliense* [6,7]. Whether improved treatment outcomes are due to decreased virulence of *M. abscessus* subsp. *massiliense* or the non-functional erm(41) gene (linked to macrolide susceptibility) is unclear. However, *M. abscessus* subsp. *massiliense* has also been associated with transmission among patients with cystic fibrosis [21]. If indeed *M. abscessus* subsp. *massiliense* is more transmissible, correct subspecies identification would also be important for infection control purposes.

While concordance between genotypic and phenotypic susceptibility testing was generally high, two functional erm(41) genes and one rrs mutation were detected in three independent *M. abscessus* subsp. *massiliense* isolates (isolates 20, 21, 31), though initial pDST showed susceptibility. Repeat pDST revealed resistance for all three isolates. This finding indicates that discrepancies between genetic and phenotypic susceptibility testing of *M. abscessus* often relate to problems of poor growth and difficulties in interpreting MICs, and not to errors in genetic assays [22].

One *M. abscessus* subsp. *bolletii* isolate with a functional erm(41) gene (isolate 34) showed intermediate resistance to Clarithromycin (MIC 4 μg/ml) on initial and susceptibility (MIC 2 μg/ml) on repeat pDST. Possible explanations include poor growth or difficulties in MIC interpretation. WGS for this isolate showed several point mutations within the erm(41) gene. Similar WGS results were observed for two other *M. abscessus* subsp. *bolletii* isolates, but the mutations were different in these strains. Whether the mutations in isolate 34 led to a dysfunctional erm(41) gene remains unknown and would need further evaluation.

Interestingly, we observed three independent *M. abscessus* subsp. *abscessus* isolates with an A2057G mutation in the rrl gene (isolates 29-31). As rrl positions 2058 and 2059 were wildtype and pDST for this isolate showed susceptibility, Repeat pDST revealed resistance for all three isolates. This finding indicates that discrepancies between genetic and phenotypic susceptibility testing of *M. abscessus* often relate to problems of poor growth and difficulties in interpreting MICs, and not to errors in genetic assays [22].

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