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# 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. *abscessus* harbour intact *erm(41)* genes. However, a T/C polymorphism at position 28 in *M. abscessus* subsp. *abscessus* 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 erm(41) gene variants, detection of point mutations in rrl and rrs, as well as determination of the T/C 28 polymorphism of erm(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, brocheoalveolar lavage, n=34), blood (n=1), 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 references.

All isolates were tested using the GenoType® NTM DR line probe assay version 1.0 (HAIN Lifescience, Nehren, Germany) according to the manufacturer's instructions. DNA was extracted as described elsewhere [16]. A senior scientist interpreted the results as per package insert. Genotypic drug resistance was determined by sequencing of the respective target genes erm(41) and rrl for macrolides, and rrs for aminoglycosides (in resistant and discordant strains only) as described previously [10,12].

Phenotypic drug susceptibility testing (pDST) was performed using the broth microdilution method according to CLSI document M24-A2 on 96-well Thermo Scientific RAPMYCOI Sensititre plates (Thermo Fisher Scientific, East Grinstead, UK) [17]. For inoculum preparation, confluent growth on 7H10 agar plates was swept with a loop and adjusted to a McFarland 0.5 turbidity standard in sterile water. Fifty microliters of this suspension were added to 10 ml of Mueller-Hinton broth and mixed; 100 µl of the final inoculum were transferred to each well of a Sensititre plate. Colony counts were performed on 7H10 plates following 3-5 days of incubation to ensure correct inoculum density ($5 \times 10^4$ to $5 \times 10^5$ cfu/mL). Plates were sealed with adhesive covers, incubated at 30°C and checked for sufficient growth in the positive control well at day 3. In case of insufficient growth, plates were reincubated and read at days 4 and 5, respectively. Additional readings were performed at days 7 and 14 to detect inducible macrolide resistance. The minimal inhibitory concentration (MIC) was defined as the lowest drug concentration at which no visual growth was observed. Clarithromycin resistance was defined as a MIC of ≥ 8 µg/ml, susceptibility as a MIC ≤ 2 µg/ml, and Amikacin resistance was defined as a MIC of ≥ 64 µg/ml, susceptibility ≤ 16 µg/ml [9,11]. Inducible resistance for Clarithromycin was defined as MIC<8 ug/ml before day 5 and MIC ≥ 8 µg/ml after 7 or 14 days of incubation, respectively.

pDST was repeated for isolates with discordant pDST results compared to the GenoType® NTM DR assay. Repeat pDST results were used for the final comparison. WGS was performed for isolates with unresolved discrepancies using Nextera library preparation and the NextSeq sequencer (Illumina, San Diego). Reads were mapped to the *M. abscessus* ATCC19977 genome (GenBank ID: NC_010397.1) with the alignment program BWA and mappings refined with the GATK and samtools toolkits. For variant detection in mapped reads, samtools and perl scripts were employed to filter for variants called by at least four reads in both forward and reverse orientation, with at least four reads calling the allele with a phred score of 20 or more, and 75% overall allele frequency. All discriminating variants were used for a concatenated sequence alignment and a maximum parsimony 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. *bolletii* CCUG 50184T and *M. massiliense* CCUG 48898T). 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).

| | | Clarithromycin | | | | | | | | | Amikacin** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No | Subsp | NTM DR | | | Sequencing | | | pDST | | | NTM DR | | pDST | |
| | | erm(41) T/C28 | rrl | Int | erm(41) T/C28 | rrl | Int | MIC d3-5 | MIC d14 | Int | rrs | Int | MIC d3-5 | Int |
| 1 | Absc | T | WT | iR | T | WT | iR | 0,25 | >16 | iR | WT | S | 8 | S |
| 2 | Absc | T | WT | iR | T | WT | iR | 0,06 | >16 | iR | WT | S | 8 | S |
| 3 | Absc | C | WT | S | C | WT | S | 0,06 | 0,5 | S | WT | S | 8 | S |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4 | Absc1 | T | WT | iR | T | WT | iR | 0,25 | >16 | iR | WT | S | 8 | S |
| 5 | #Absc | T | A2058G | R | T | A2058G | R | 1 | >16 | iR | A1408G | R | >64 | R |
| 6 | Absc | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 16 | S |
| 7 | Absc | T | WT | iR | T | WT | iR | 0,06 | >16 | iR | WT | S | 4 | S |
| 8 | Absc | T | WT | iR | T | WT | iR | 0,25 | >16 | iR | WT | S | 8 | S |
| 9 | Absc | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 8 | S |
| 10 | Absc | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 16 | S |
| 11 | Absc | T | WT | iR | T | WT | iR | 0,06 | 16 | iR | WT | S | 8 | S |
| 12 | Absc2 | C | WT | S | C | WT | S | 0,06 | 0.12 | S | WT | S | 4 | S |
| 13 | Absc | T | A2058C | R | T | A2058C | R | >16 | >16 | R | A1408G | R | >64 | R |
| 14 | Absc | T | WT | iR | T | WT | iR | 0,5 | >16 | iR | WT | S | 8 | S |
| 15 | Absc | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 8 | S |
| 16 | Absc | T | WT | iR | T | WT | iR | 0,06 | >16 | iR | WT | S | 8 | S |
| 17 | Absc | T | WT | iR | T | WT | iR | 0,25 | >16 | iR | WT | S | 8 | S |
| 18 | Absc | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 8 | S |
| 19 | Absc | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 8 | S |
| 20 | #Absc | T | WT | iR | T | WT | iR | 0,25 | 1 | S | WT | S | 8 | S |
| 21 | #Absc[1] | T | WT | iR | T | WT | iR | 0,12 | 2 | S | WT | S | 8 | S |
| 22 | Absc | T | WT | iR | T | WT | iR | 0,06 | >16 | iR | WT | S | 4 | S |
| 23 | Absc | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 4 | S |
| 24 | Absc | T | WT | iR | T | WT | iR | 0,06 | >16 | iR | WT | S | 4 | S |
| 25 | Absc | T | WT | iR | T | WT | iR | 0,5 | >16 | iR | WT | S | 8 | S |
| 26 | Absc | C | WT | S | C | WT | S | 0,06 | 0.5 | S | WT | S | 4 | S |
| 27 | Absc | C | WT | S | C | WT | S | 0,06 | 0.25 | S | WT | S | 8 | S |
| 28 | Absc | T | WT | iR | T | WT | iR | 0,06 | >16 | iR | WT | S | 4 | S |
| 29 | Absc2 | C | WT | S | C | A2057C | ? | 0,06 | 1 | S | WT | S | 8 | S |
| 30 | #Absc | T | WT | iR | T | A2057G | ? | 0,12 | >16 | iR | WT | S | >64 | R |
| 31 | #Absc | T | WT | iR | T | A2057G | ? | 0,12 | >16 | iR | A1408G | R | 8 | S |
| 32 | #Boll | T | WT | iR | T | WT | iR | 0,5 | >16 | iR | WT | S | 64 | R |
| 33 | Boll | T | WT | iR | T | WT | iR | 0,25 | >16 | iR | WT | S | 16 | S |
| 34 | #Boll | T | WT | iR | T | WT | iR | 0,06 | 4 | I | WT | S | 8 | S |
| 35 | Boll | T | WT | iR | T | WT | iR | 0,06 | >16 | iR | WT | S | 8 | S |
| 36 | Boll | T | WT | iR | T | WT | iR | 0,12 | >16 | iR | WT | S | 2 | S |
| 37 | Boll | T | WT | iR | T | WT | iR | 0,5 | >16 | iR | WT | S | 2 | S |
| 38 | Massil* | T | WT | S | T | WT | S | 0,12 | 0.5 | S | WT | S | 8 | S |
| 39 | Massil* | T | WT | S | T | WT | S | 0,12 | 0.5 | S | WT | S | 8 | S |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 40 | Massil* | T | WT | S | T | WT | S | 0,06 | 0.25 | S | WT | S | 8 | S |
| 41 | Massil* | T | WT | S | T | WT | S | 0,06 | 1 | S | WT | S | 4 | S |
| 42 | Massil | T | WT | S | T | WT | S | 0,06 | 0,06 | S | WT | S | 16 | S |
| 43 | Massil | T | A2058C | R | T | A2058C | R | >16 | >16 | R | A1408G | R | >64 | R |
| 44 | Massil | T | A2058G | R | T | A2058G | R | >16 | >16 | R | WT | S | 8 | S |
| 45 | Massil | T | A2058C | R | T | A2058C | R | >16 | >16 | R | A1408G | R | >64 | R |
| 46 | Massil | T | A2058C | R | T | A2058C | R | >16 | >16 | R | A1408G | R | >64 | R |
| 47 | Massil | T | A2058C | R | T | A2058C | R | >16 | >16 | R | A1408G | R | >64 | R |
| 48 | Massil | T | WT | S | T | WT | S | 0,06 | 0.5 | S | WT | S | 8 | S |

*: Discordances in subspecies differentiation between Sanger sequencing (*M. abscessus* subsp. *abscessus*) and GenoType® NTM DR (*M. abscessus* subsp. *massiliense*);
**: The rrs gene was sequenced for all isolates showing resistance to Amikacin (Mut1–A1408G) or discordance between pDST and GenoType® NTM DR results.
1,2: same patient;
#: Discordances in initial pDST results (Clarithromycin and/or Amikacin).

**Repeat pDST for respective isolates:**

Clarithromycin, MIC d3-5/d14, µg/ml, Int: Isolate No. 5: 1/>16, iR; isolate No. 20: 0.25/>16, iR; isolate No.21: 0.12/>16, iR; isolate No. 34 0.06/2, S

Amikacin, MIC d3-5, µg/ml, Int: Isolate No. 30: >64, R; isolate No. 31: >64, R; isolate No 32: >64, R.

**Table 1:** GenoType® NTM DR, sequencing and first phenotypic drug susceptibility testing (pDST) results for clarithromycin and amikacin.

Subspecies identification by Sanger sequencing revealed 35 *M. abscessus* subsp. *abscessus*, six *M. abscessus* subsp. *bolletii* 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. *bolletii,* 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 rrs position 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 rrs position 1408 (in isolates showing resistance to Amikacin or discordant pDST results). 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, differenciating 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. *abscessus* 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 Clarithromycin did not show resistance on days 3-5, genotypic and phenotypic test results were considered concordant. However, this rarely occurring mutation has previously been described as conferring medium to low level resistance to Clarithromycin in *M. abscessus* (MIC 8 µg/ml) [13,23]. Further studies are needed to reveal the significance of this point mutation in clinical *M. abscessus* isolates. For one of the isolates (isolate 29), another isolate from the same patient didn't show this mutation (isolate 12), which could be indicative for a mixed *M. abscessus* population.

Two isolates (isolates 30 and 32) without evidence of any mutations in the rrs gene as determined by the GenoType® NTM DR assay and Sanger sequencing were repeatedly resistant to Amikacin by pDST. WGS revealed point mutations at positions 1305 and 1306 of the rrs gene in one of the two isolates. Whether this mutation confers resistance to aminoglycosides is currently unknown. WGS did not find any mutation of the rrs gene in the second isolate. Other mechanisms such as changes in cell wall permeability might lead to Amikacin resistance and explain these results [24].

Macrolides and Amikacin are the cornerstones of *M. abscessus* treatment regimens if the isolate is susceptible [1,5]. Furthermore, macrolides are often the only oral therapeutic option for *M. abscessus* disease. As the recommended treatment duration is 12 months following culture conversion, adverse events associated with therapy are common [1,5]. In our hands, the GenoType® NTM DR allowed rapid subspecies identification and accurate determination of mutations conferring resistance to Clarithromycin and Amikacin. As such, the assay can readily be implemented in a routine laboratory. The clinical relevance of the results and the potential advantages over pDST remain a matter of debate. However, early knowledge about acquired aminoglycoside or macrolide resistance allows rapid and more rational design of therapeutic regimens, prevention of unnecessary drug toxicity and more transparent patient communication. The rapidity of the results compared to pDST might be an advantage for a certain patient population. We believe this actively enhances patient experience and control in decision-making [25].

An inherent drawback of line probe assays such as the GenoType® NTM DR is their limitation to testing known mutations conferring phenotypic resistance. In contrast, pDST allows detection of resistance regardless of the underlying mechanism. This is particularly important when determining susceptibility for *M. abscessus* as resistance is not exclusively due to mutations, but may result from other mechanisms such as upregulated drug export systems or decreased cell wall permeability [26]. On the other hand, pDST interpretation can be difficult for rapidly-growing mycobacteria as poor growth can lead to falsely susceptible results [22]. In this case GenoType® NTM DR can help to improve the interpretation of pDST results by supporting or refuting them. In fact, parallel susceptibility testing using pDST and the GenoType® NTM DR appears to be useful to ensure consistency across methods.

This study has several limitations. The sample size (48 isolates from 46 patients) was small. Isolates sent to a National Reference Laboratory are likely to be pre-selected and therefore results might not be generalizable. Clinical information was available for few isolates, precluding any meaningful analysis of clinical data. Diagnostic gold standards for *M. abscessus* are lacking as both genetic and phenotypic susceptibility testing methods possess the intrinsic limitations mentioned above. Thus sensitivity and specificity as well as predictive values of the GenoType® NTM DR were not assessed.

Our current knowledge of *M. abscessus* disease is incomplete. New molecular diagnostic tools such as the GenoType® NTM DR will allow rapid and accurate subspecies differentiation and determination of genetic resistance markers. Although they cannot replace pDST, they can support the interpretation of pDST data, guide treatment, and enhance our understanding of *M. abscessus* as an emerging pathogen. Finally, a reliable diagnostic workflow integrating both genotypic and phenotypic methods will support the execution of urgently needed clinical studies correlating *in vitro* drug susceptibility data to clinical outcome.

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