





## Introduction

The fast-growing *Mycobacterium abscessus* complex is an emerging opportunistic human pathogen worldwide [1]. It includes three subspecies: *M. abscessus* subspecies *abscessus*, *M. abscessus* subsp. *massiliense*, and *M. abscessus* subsp. *bolletii* [2]. The most frequent clinical manifestation of *M. abscessus* complex infection is chronic lung infection, usually in elderly women with bronchiectasis and in young adults with cystic fibrosis (CF) [3].

*M. abscessus* carries or acquires inducible resistance to macrolides (e.g. clarithromycin) and aminoglycosides (e.g. amikacin) used to treat pulmonary infections. Resistance to macrolides can be: i) constitutive (associated with point mutations at position A2058 and A2059 of the *rrl* gene, a macrolide target), in all subspecies; and ii) inducible (associated with the *erm(41)* gene T28 sequevar), only in *M. abscessus* subsp. *abscessus* and *bolletii* [4]. A single 16S ribosomal RNA (rRNA) substitution at position 1408 in the *rrs* gene causes high-level aminoglycoside resistance [4, 5]. Rapid molecular diagnosis is essential for subspecies identification and determination of the adequate antimicrobial therapy, and is now recommended in patients with CF [3, 6].

The recently commercialized GenoType NTM-DR test (NTM-DR) (Hain, Lifescience, Nehren, Germany) allows the rapid *M. abscessus* subspecies identification and simultaneously the detection of resistance to macrolides and aminoglycosides [7–9].

This study wanted to 1) determine NTM-DR performance for subspecies identification in *M. abscessus* complex isolates (compared with MLST), and 2) evaluate NTM-DR ability to detect clarithromycin and amikacin resistance (compared with phenotypic and sequencing analyses).

## Materials and methods

### Patients and mycobacteria isolates

For this study, 115 *M. abscessus* complex isolates (1 isolate/patient) were randomly selected among the 176 isolates (1 isolate/patient) from respiratory samples of 176 different patients with CF or other respiratory diseases stored at the Microbiology Laboratory, University Hospital, Montpellier (France). All *M. abscessus* complex isolates came from six University Hospitals in France (Brest, Bordeaux, Montpellier, Nantes, Rouen, and Toulouse), including a Reference Centre for CF.

### Molecular analysis and drug susceptibility testing

Total DNA was extracted using GenoLyse v2.0 (Hain Lifescience, Nehren, Germany). Isolates were assigned to the *M. abscessus* complex using the multiplex GenoType Mycobacterium CM assay (Hain Lifescience Nehren, Germany), and subspecies were identified with the GenoType NTM-DR Kit (Hain Lifescience, Nehren, Germany), the index test, according to the manufacturer's recommendations. All isolates were also characterized by multilocus sequence typing (MLST), based on eight housekeeping genes (*argH*, *cya*, *glpK*, *gnd*, *murC*, *pgm*, *pta*, and *purH*), as previously described [10]. *M. abscessus* complex subspecies, allele and sequence type were identified using the *M. abscessus* MLST database (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Myco-abscessus>).

Resistance was assessed using the GenoType NTM-DR Kit and by phenotypic drug susceptibility testing (DST), the reference method [11]. The Minimum Inhibitory Concentrations (MICs) of clarithromycin and amikacin were evaluated using the reference broth microdilution method with Sensititre RAPMYCO microplates (Trek Diagnosis Systems). Susceptibility and resistance were assessed according to the CLSI recommendations. High-level



clarithromycin resistance was defined by a MIC  $\geq 8$   $\mu\text{g/ml}$  at day 5. Inducible resistance was defined by an increase in clarithromycin MIC from  $\leq 2$   $\mu\text{g/ml}$  at day 5 to  $\geq 8$   $\mu\text{g/ml}$  at day 14. Aminoglycoside resistance was defined by MIC values  $\geq 64$   $\mu\text{g/ml}$  [11]. Mutations in the *erm* (41), *rrl* and *rrs* genes were identified by PCR and sequencing, as previously described [12, 13].

NTM-DR detects heteroresistance in mixed populations of drug-susceptible and -resistant mycobacteria and shows the simultaneously the presence of wild type (WT) and mutant (MUT) bands.

## Statistical analyses

Based on an expected NTM-DR assay sensitivity of 96% for subspecies identification (100% and 92% according to the manufacturer and Kehrmann et al. [7], respectively), 115 of the 176 isolates (60 *M. abscessus* subsp. *abscessus*, 47 *M. M. abscessus* subsp. *massiliense*, and 8 *M. abscessus* subsp. *bolletii*) were needed to assess the test sensitivity with a precision of  $\pm 5\%$ .

## Results

The agreement between NTM-DR and MLST results for subspecies identification was 100% (60/60) for *M. abscessus* subsp. *abscessus*, 100% (47/47) for *M. abscessus* subsp. *massiliense*, and 100% (8/8) for *M. abscessus* subsp. *bolletii*. NTM-DR sensitivity and specificity for *M. abscessus* subsp. *abscessus* identification were 100% [95% CI: 96.2–100] and 100% [95% CI: 93.5–100].

Both NTM-DR and DST identified 59/115 (51.3%) isolates as resistant to clarithromycin: 12 ( $n = 7$  *M. abscessus* subsp. *abscessus* and  $n = 5$  *M. abscessus* subsp. *massiliense*) with high resistance level and 47 ( $n = 39$  *M. abscessus* subsp. *abscessus* and  $n = 8$  *M. abscessus* subsp. *bolletii*) with inducible resistance (S1 Table). Both techniques identified six (5.2%, 6/115) isolates resistant to aminoglycosides ( $n = 4$  *M. abscessus* subsp. *abscessus* and  $n = 2$  *M. abscessus* subsp. *massiliense*) (S1 Table). Agreement was total between NTM-DR and DST results for clarithromycin and aminoglycoside resistance.

NTM-DR identified *rrl* mutations associated with high level of resistance in the 12 isolates with high resistance to clarithromycin ( $n = 4$  A2058C,  $n = 5$  A2058G, and  $n = 3$  A2059G *rrl* mutation, corresponding to the positive MUT1, MTU2 and MUT4 bands of NTM-DR, respectively, (S1 Table). Among the 12 clarithromycin-resistant *M. abscessus* subsp. *abscessus* isolates, 11 showed a *rrl* profile without WT band and one presented a heterogeneous pattern (one WT and one MUT1 band), suggesting the presence of a mixed population. In all 47 isolates with inducible clarithromycin resistance, NTM-DR detected a specific *erm* (41) T28 sequevar band and a WT band in the *rrl* gene. Finally, all 56 isolates susceptible to clarithromycin by DST were susceptible also by NTM-DR (*erm*(41) C28 sequevar band and WT band in the *rrl* gene) (S1 Table).

The six isolates with aminoglycoside resistance displayed the positive MUT1 band by NTM-DR. Sequencing confirmed the presence of the A1408G *rrs* mutation in these isolates. The 109 (94.8%) aminoglycoside-susceptible isolates showed a WT band by NTM-DR and WT *rrs* gene sequence (S1 Table). Overall, NTM-DR and sequencing showed 100% agreement. The concordance between NTM-DR and DST was 100% for clarithromycin and amikacin. NTM-DR sensitivity for the detection of clarithromycin and amikacin resistance was also 100%.

## Discussion

Our study showed an excellent concordance (100%) between NTM-DR and MLST results concerning subspecies identification, and confirmed the high sensitivity of NTM-DR for detecting



acquired resistance. This is higher than what reported by a recent study on 50 *M. abscessus* complex isolates (92% concordance between NTM-DR and gene sequencing for subspecies identification) [7, 14, 15]. The excellent results observed confirmed that the NTM-DR assay seems a discriminative method for *M. abscessus* complex subspecies identification [7, 9, 16].

Our study showed that NTM-DR can detect acquired resistance with high sensitivity. This test also easily detects heteroresistance, not always possible by sequencing [8]. This is crucial for treatment decision-making, and it would be relevant to evaluate *in vitro* NTM-DR sensitivity for heteroresistance detection in mixed populations of drug-susceptible and -resistant mycobacteria.

Although this is not the case for our sample, it has been reported that some isolates with phenotypic resistance to macrolides and aminoglycoside do not carry mutations in the *rrl* and *rrs* genes. This implies the existence of other resistance mechanisms that cannot be detected by the NTM-DR and other commercial kits [7, 8]. For example, a modification (direct repeat 18-bp insertion in *rpIV*) in the ribosomal protein L4 has been recently associated with resistance to macrolides [17].

## Conclusion

The excellent NTM-DR performance indicates that this test is a robust and complementary tool to MLST and broth microdilution for subspecies identification and detection of clarithromycin and amikacin resistance in *M. abscessus* complex. NTM-DR could be used for routine testing, particularly in patients with and without CF and *M. abscessus* complex lung infections.

## Supporting information

**S1 Table.**  
(XLSX)

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## References

1. Lee M.R., et al., Mycobacterium abscessus Complex Infections in Humans. *Emerg Infect Dis*, 2015. 21 (9): p. 1638–46. <https://doi.org/10.3201/2109.141634> PMID: 26295364
2. Floto R.A., et al., US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. *Thorax*, 2016. 71 Suppl 1: p. i1–22.



3. Griffith D.E., et al., *Mycobacterium abscessus*. "Pleased to meet you, hope you guess my name. . .". *Ann Am Thorac Soc*, 2015. 12(3): p. 436–9. <https://doi.org/10.1513/AnnalsATS.201501-015OI> PMID: 25643064
4. Bastian S., et al., Assessment of clarithromycin susceptibility in strains belonging to the *Mycobacterium abscessus* group by *erm*(41) and *rfl* sequencing. *Antimicrob Agents Chemother*, 2011. 55(2): p. 775–81. <https://doi.org/10.1128/AAC.00861-10> PMID: 21135185
5. Prammananan T., et al., A single 16S ribosomal RNA substitution is responsible for resistance to amikacin and other 2-deoxystreptamine aminoglycosides in *Mycobacterium abscessus* and *Mycobacterium chelonae*. *J Infect Dis*, 1998. 177(6): p. 1573–81. <https://doi.org/10.1086/515328> PMID: 9607835
6. Floto R.A., et al., US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis: executive summary. *Thorax*, 2016. 71(1): p. 88–90. <https://doi.org/10.1136/thoraxjnl-2015-207983> PMID: 26678435
7. Kehrmann J., et al., GenoType NTM-DR for Identifying *Mycobacterium abscessus* Subspecies and Determining Molecular Resistance. *J Clin Microbiol*, 2016. 54(6): p. 1653–1655. <https://doi.org/10.1128/JCM.00147-16> PMID: 27030487
8. Mougari F., et al., Evaluation of the new GenoType NTM-DR kit for the molecular detection of antimicrobial resistance in non-tuberculous mycobacteria. *J Antimicrob Chemother*, 2017. 72(6): p. 1669–1677. <https://doi.org/10.1093/jac/dkx021> PMID: 28333340
9. Huh HJ; et al., GenoType NTM-DR Performance Evaluation for Identification of *Mycobacterium avium* Complex and *Mycobacterium abscessus* and Determination of Clarithromycin and Amikacin Resistance. *J Clin Microbiol*, 2019. 57(8).
10. Macheras E., et al., Multilocus sequence analysis and *rpoB* sequencing of *Mycobacterium abscessus* (sensu lato) strains. *J Clin Microbiol*, 2011. 49(2): p. 491–9. <https://doi.org/10.1128/JCM.01274-10> PMID: 21106786
11. Clinical and Laboratory Standards Institute [CLSI] (2018). *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes*, 3rd Edn. Wayne, PA: CLSI.
12. Maurer F.P., et al., Acquisition of clarithromycin resistance mutations in the 23S rRNA gene of *Mycobacterium abscessus* in the presence of inducible *erm*(41). *J Antimicrob Chemother*, 2012. 67(11): p. 2606–11. <https://doi.org/10.1093/jac/dks279> PMID: 22833642
13. Nash K.A., Brown-Elliott B.A., and Wallace R.J. Jr., A novel gene, *erm*(41), confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. *Antimicrob Agents Chemother*, 2009. 53(4): p. 1367–76. <https://doi.org/10.1128/AAC.01275-08> PMID: 19171799
14. Ruger K., et al., Characterization of rough and smooth morphotypes of *Mycobacterium abscessus* isolates from clinical specimens. *J Clin Microbiol*, 2014. 52(1): p. 244–50. <https://doi.org/10.1128/JCM.01249-13> PMID: 24197890
15. Macheras E., et al., Inaccuracy of single-target sequencing for discriminating species of the *Mycobacterium abscessus* group. *J Clin Microbiol*, 2009. 47(8): p. 2596–600. <https://doi.org/10.1128/JCM.00037-09> PMID: 19515839
16. Macheras E., et al., Multilocus sequence typing scheme for the *Mycobacterium abscessus* complex. *Res Microbiol*, 2014. 165(2): p. 82–90. <https://doi.org/10.1016/j.resmic.2013.12.003> PMID: 24384536
17. Mougari F., et al., Selection of Resistance to Clarithromycin in *Mycobacterium abscessus* Subspecies. *Antimicrob Agents Chemother*, 2017. 61(1).