Performance of GenoType NTM-DR kit for identification of Mycobacterium abscessus subspecies and detection of antibiotic resistance

Bouzinbi N.1, Bertolotti T.1, Aberkane S.1, Appelgren A.1, Jean-Pierre H.1, Panteix G.3, Marcy O and Godreuil S.1,2
Department of bacteriology & virology, CHU Arnaud de Villeneuve 34295 Montpellier France1, Department of Microbiology, MIVEGEC UMR IRD 224-CNRS 52902, Biomiss 1, 17-19 Avenue Tony Garnier, 69357 Lyon France

BACKGROUND

Mycobacterium abscessus (MA) is a rapid growing non tuberculous bacteria (RGM) involved in pulmonary, soft tissues and disseminated infections. It can be viewed as a complex, comprising 3 subspecies: Mycobacterium abscessus stricto sensu, Mycobacterium subspp. massiliense and Mycobacterium subspp. Bolletii. Because subspecies in M. abscessus complex differ in antibiotic resistance and treatment response, a rapid molecular diagnosis is essential, and now recommended from CF patients, for subspecies identification and for guiding adequate antimicrobial therapy. The recently commercialized GenoType NTM-DR tests (Hain, Lifescience, Nehren, Germany) (3) is a new line probe assay that allows rapid simultaneous detection and differentiation of M. abscessus subspecies and detection of the resistance to macrolides and aminoglycosides from mycobacterial isolates. The aim of this study was to determine the performance of the GenoType NTM-DR assay for subspecies identification in M. abscessus complex isolates. The secondary objective was to compare the molecular resistance results of M. abscessus complex isolates from NTM-DR assay with results of phenotypic antibiotic susceptibility testing of clarithromycin and amikacin.

MATERIAL & METHODS

- The hundred seventy six M. abscessus complex isolates from respiratory sample of CF and non-CF patients obtained from collection of the Microbiology Laboratory, University Hospital, Montpellier (France) between January 2008 and December 2015. Three M. abscessus complex reference isolates (M. abscessus CIP 104536 T, M. massiliense CIP 108297 T, M. bolletii CIP 108541 T). With an expected sensitivity of the NTM-DR assay of 97%, 86 (86/176) M. abscessus complex isolates (45 M. abscessus (45/98), 35 M. massiliense (35/68) and 6 M. bolletii (6/10) were needed to assess the sensitivity with a precision of +/-5%.

- M. abscessus complex species identification. Total DNA was extracted using Genolyse v 2.0 (Hain Lifescience, Nehren, Germany) and the boiling method. Based on the commercial multiplex line-probe assay Geno-Type Mycobacterium CM (Hain Lifescience Nehren, Germany), all 176 isolates were assigned to the species M. abscessus complex. All isolates were also characterized by using the MLST method, based on seven housekeeping genes (argH, cya, glpK, gnd, murC, pta and purH) as described by Macheras et al.

- MICs of clarithromycin and amikacin were obtained by the reference microdilution method using Sensititre RAPMAC microplates (Trek Diagnosis Systems).

- GenoType NTM-DR testing. The NTM-DR kit (Hain Lifescience, Nehren, Germany) was performed according to the manufacturer’s recommendations.

RESULTS


- 77 of the strains (96, 25 %) were correctly identified to the subspecies level with the NTM-DR kit.

- 3 of the strains were mistakenly identified: one M. abscessus subspecies abscessus was identified as M. bolletii, one MA subspecies abscessus as M. massiliense and one M. massiliense as MA subspecies abscessus.

- An important number of strains had a macrolide resistance: 47/81 (58 %). The vast majority of the non-macrolide resistant strains were the M. abscessus sub. massiliense strains: 22/27 (81,4 %), confirming the broth microdilution results.

- 5 strains were all M. abscessus sub. abscessus with a C on position 28 of the erm(41) gene; 4 of the macrolide resistant strains harbored a type 2 mutation on the rrl gene.

- No aminoglycoside resistant strain were detected.

DISCUSSIO

The NTM-DR kit provides a rapid (< than 5 hours), cost-efficient solution to the optimization of the treatment for M. abscessus infections, with a solid identification rate between the subspecies and the characterization of the principal antibiotic resistance: macrolides and aminoglycosides. It can palliate the time consuming (14 days of incubation) and liable broth microdilution method for antibiotic resistance detection. A variation in the genomic regions of the primers and the probes can explain the few mistake, since this technique rely on hybridization. To our knowledge, this is the first study implementing a proper randomized essay to test the NTM-DR kit. In conclusion, this kit can definitely be implemented in hospital mycobacterial routine in its current state, with the use of the MALDI-TOF as a complementary tool since It has seen tremendous improvement in the recent years of the MA subspecies identification in the recent years.