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Mycobacteriology

Performance of Vitek MS in identifying nontuberculous mycobacteria from MGIT liquid medium and Lowenstein–Jensen solid medium

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ABSTRACT

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry is a fast and inexpensive method for bacterial identification. The aim of this study was to analyze the performance of Vitek MS in identifying 160 nontuberculous mycobacterial isolates of 24 species from Lowenstein–Jensen solid medium and BACTEC MGIT 960 liquid medium using a bead-based method. The system correctly identified 76.9% of the isolates (123 of 160) cultivated on solid medium and 76.9% (123 of 160) of positive liquid cultures. None of the isolates included in the study was misidentified. Although the overall performance of Vitek MS with the SARAMIS 4.12 database was comparable in identifying mycobacterial species grown on solid medium and in liquid medium, the identification rate varied notably between the various species analyzed, which currently limits the utility for identification in routine diagnostics for some species.

1. Introduction

Nontuberculous mycobacteria (NTM) are widely distributed in the environment, and the number of pulmonary and extrapulmonary infections caused by NTM is increasing worldwide (McGrath and Anderson, 2007; Tortoli, 2014). Identifying these bacteria at the species level may be important for therapeutic and epidemiologic reasons. Identification is currently achieved predominantly with molecular methods, such as DNA probes (Lebrun et al., 1992) and single-phase reverse hybridization technology (Richter et al., 2006; Tortoli et al., 2003), which identify the most frequently isolated species. Gene sequencing, especially of the 16S ribosomal RNA (rRNA) gene, is the reference method for mycobacterial identification (Tortoli, 2010). However, some species cannot be easily discriminated by 16S rRNA gene sequencing because of the high sequence similarities within this gene, so that sequencing of other genomic regions may be necessary for their unambiguous differentiation.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has become a powerful method for bacterial identification in microbiological diagnostics (Charnot-Katsikas et al., 2014; Clark et al., 2013; Fang et al., 2012; Rand and Delano, 2014). This technique enables bacterial identification by generating and deciphering the spectral fingerprints produced by extracted molecules (Marvin et al., 2003) and is a rapid and inexpensive alternative method that facilitates mycobacterial identification. However, in contrast to most other bacteria, which can be reliably identified by whole cell analysis with MALDI-TOF MS, the notable stability of mycobacteria and their cell wall characteristics complicate MALDI-TOF MS profiling and require preparation before analysis if high-quality spectra are to be generated (Balazova et al., 2014; El Khechine et al., 2011). In contrast to other bacteria, the mycobacterial cell wall is characterized by a high proportion of mycolic acids, resulting in a waxy, hydrophobic structure. For disrupting clumped cells and breaking open cell envelopes, special processing is required before MALDI-TOF MS analysis (Balazova et al., 2014; Patel, 2015). Three commercial MALDI-TOF MS systems are available for bacterial identification: Maldi Biotyper (Bruker Daltronics, Bremen, Germany), Vitek MS (bioMérieux, Marcy l'Etoile, France), and Andromas MALDI-TOF MS (Andromas, Paris, France). The database, which is crucial for the correct identification of species, differs among the 3 MALDI-TOF MS systems.

We aimed to study the performance of Vitek MS with the current SARAMIS 4.12 research use only (RUO) database in identifying 160 NTM isolates, including 24 separate species cultured in the commonly used BACTEC mycobacteria growth indicator tube (MGIT) 960 liquid medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and on Lowenstein–Jensen solid medium (Oxoid, Basingstoke, Hampshire, UK). Because mycobacterial liquid cultures usually become positive earlier than solid cultures, MALDI-TOF MS identification of mycobacteria grown in liquid medium shortens the time to NTM identification and, therefore, could be important for early clinical diagnosis.

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2. Materials and methods

2.1. Isolates and cultures

In this study, we analyzed 160 NTM isolates, including 6 reference strains and 154 clinical isolates. Clinical isolates of patients of the University Hospital Essen and 30 *Mycobacterium abscessus* isolates, provided by the department of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, were used in this study. Isolates were recultured from frozen stocks stored in the mycobacterial strain collection of the University Hospital Essen in liquid BACTEC MGIT 960 medium (Becton, Dickinson and Company) and on solid Lowenstein–Jensen medium (Oxoid) at 37 °C. For diagnostic purposes, clinical isolates had primarily been identified by 1 of the following methods: GenoType Mycobacterium CM, GenoType Mycobacterium AS (Hain Lifescience, Nehren, Germany), or AccuProbe assays (bioMérieux). Identification of the clinical isolates used in this study was additionally confirmed by 16S rRNA sequencing, which is considered to be the reference method for mycobacterial identification. No identification discrepancies between the different molecular identification methods occurred for the isolates, used in this study.

2.2. Mycobacterial preparation protocol

The mycobacterial preparation protocols suggested by bioMérieux were used with the following modifications: in order to improve the removal of remnants of the medium, a second washing step in 500-μL 70% ethanol was added for the liquid medium preparation procedure, and glass beads, 1 mm instead of 0.5 mm in diameter, were used with both protocols for cell disruption.

2.2.1. Liquid medium

First, 1.8 mL of liquid medium from the bottom of the positive MGIT culture was pipetted into a centrifuge vial and centrifuged at 6000 × g for 10 min. The supernatant was discarded, and the pellet was resuspended in 500 μL 70% ethanol. After centrifugation at 6000 × g for 10 min, the pellet was resuspended in another 500 μL 70% ethanol and transferred into a centrifuge vial containing 200 μL of glass beads (Roth, Karlsruhe, Germany), each 1 mm in diameter. This second ethanol washing step was added to the original bioMérieux protocol. The cells were disrupted with a vortex (IKA MS3 digital; Sigma-Aldrich, St Louis, MO, USA) for 15 min and then incubated at room temperature for 10 min. The suspension was transferred into a sterile tube and centrifuged for 2 min at 18,000 × g. The pellet was resuspended with 10 μL of 70% formic acid. After the addition of 10 μL of 100% acetonitrile, the suspension was briefly vortexed and then centrifuged at 18,000 × g for 2 min. Next, 1 μL of the supernatant was pipetted onto the MALDI-TOF target slide. The samples were dried at room temperature, and 1 μL α-cyano-4-hydroxy cinnamic acid (CHCA) matrix solution (bioMérieux, product number 411071) was added. Samples were analyzed in quadruplicate after drying at room temperature.

2.2.2. Solid medium

A 1 μL loopful of mycobacterial biomass was transferred into a 2-mL microcentrifuge vial containing 500 μL of 70% ethanol and approximately 200 μL of glass beads (Roth), each 1 mm in diameter. After mechanical disruption by vortexing for 15 min, vials were incubated at room temperature for 10 min to complete inactivation. The suspension was transferred into a sterile tube and centrifuged at 18,000 × g for 2 min, after which the ethanol supernatant was removed. The pellet was resuspended in 10 μL of 70% formic acid. An additional 10 μL of 100% acetonitrile was added, and the solution was briefly vortexed. After centrifugation at 18,000 × g for 2 min, 1 μL of the supernatant was pipetted onto the MALDI-TOF target slide. After the samples had been dried at room temperature, 1 μL CHCA matrix solution was added (bioMérieux, product number 411071). Having dried at room temperature, the samples were analyzed in quadruplicate.

2.3. Vitek MS identification

The samples were analyzed with the Vitek MS Plus system using the SARAMIS 4.12 RUO database, which includes a higher number of mycobacterial species compared to the in vitro diagnostics (IVD) database. While the IVD database includes 10 mycobacterial species, the VITEK MS Plus system with the SARAMIS 4.12 database represents an extended database including 45 mycobacterial species. The results were generated from automated comparison of sample peak lists with the SuperSpectra in the database. All of the species used in this study were included in the database. The molecular profile of each isolate was analyzed with an m/z (molecular mass in Daltons divided by charge in units of the elementary charge) of 2000–20,000. The identification match was indicated by a confidence level (CL) ranging from 0% to 99.9%. The spots with a CL higher than 98% were classified as high-confidence identification; those with a CL of 85–98% were classified as medium-confidence identification; and those with a CL higher than 75% but lower than 85% were classified as low-confidence identification. Spots that were less than 75% similar to any of the SuperSpectra were classified as unidentified, which is the default assessment of the SuperSpectra analysis method of VITEK MS Plus system. If “no spectrum” was reported, the mycobacterial preparation analysis was performed one more time. If the result of the repeated analysis was “no spectrum”, this was assessed as unidentified. Misidentification was defined as an assignment of an isolate to a wrong species.

3. Results

We analyzed 160 NTM isolates comprising 24 separate mycobacterial species taken from positive Lowenstein–Jensen solid medium and MGIT liquid medium cultures. At the species level, Vitek MS correctly identified 123 (76.9%) of the 160 isolates from both, Lowenstein–Jensen solid medium and MGIT liquid medium (Table 1). The CL for 97 (78.9%) cultures from solid medium and for 85 (69.1%) of the 123 identified cultures from liquid medium was classified as high (>98%); the CL for 22 (17.9%) cultures from solid medium and for 18 (14.6%) cultures from liquid medium was classified as medium (85–98%); and the CL for 4 (3.3%) cultures from solid medium and for 20 (16.3%) cultures from liquid medium was classified as low (>75% to <85%). Eleven solid cultures and 6 liquid cultures of a total of 14 different isolates were prepared again because “no spectrum” was reported. These repeated preparations contained 3 *M. malmoense*, 3 *M. kansasii*, 3 *M. celatum*, 2 *M. avium*, 2 *M. scrofulaceum*, and 1 *M. avium* isolates. Of those repeats, 4 cultures were correctly identified including 1 *M. avium* liquid and 1 *M. avium*, 1 *M. scrofulaceum*, and 1 *M. fortuitum* solid culture.

For all isolates, Vitek MS either named a single species with a certain CL or no identification could be achieved. Regarding the cultures from solid medium, the technique correctly identified all isolates of *M. abscessus* (33 of 33 isolates were identified correctly), *M. avium* (23 of 23), *M. asiaticum* (1/1), *M. chelonae* (1/1), *M. scrofulaceum* (5/5), *M. peregrinum* (3/3), *M. simiae* (4/4), *M. phlei* (3/3), *M. smegmatis* (5/5), *M. szulgai* (4/4), and *M. vaccae* (1/1) (Table 1). None of the isolates included in the study was misidentified. *M. porcinum*, which belongs to the *M. fortuitum* complex, was identified as *M. fortuitum*, which is also indicated in the bioMérieux manual; therefore, this identification was assessed as correct. The identification rate of some other clinically relevant species, including *M. kansasii* (4/12), *M. malmoense* (1/7), and *M. xenopi* (3/9), was less than 50%.

There were no significant differences in the identification rates for the same species cultivated either in MGIT liquid medium or on solid medium. However, the percentage of isolates identified with a low CL was higher from liquid medium (20/123) compared to solid medium