

Short Communication

MALDI-TOF MS improves routine identification of non-fermenting Gram negative isolates from cystic fibrosis patients

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Abstract

Identification of non-fermenting Gram-negative bacteria (NFGNB) from cystic fibrosis (CF) patients is often limited. A collection of stored NFGNB isolates (n=182) recovered from CF patients over a 15 year period was examined. The routinely reported identification during this period was compared with that obtained by MALDI-TOF MS. Isolates giving discrepant identification at the genus level were further analyzed by 16S rDNA sequencing. The MALDI-TOF MS system identified 94% of the isolates, including *Burkholderia cepacia* and *Pandoraea* spp. isolates, the latter previously misidentified as other NFGNB by conventional microbiological methods. Lack of identification by MALDI-TOF MS was associated with the absence of entries in the database.

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Non-fermenting Gram-negative bacteria (NFGNB) are environmental organisms that can cause opportunistic severe infections, especially in immunocompromised patients. In cystic fibrosis (CF) patients, the main causes of morbidity and mortality are the decline in pulmonary function secondary to pathogenic colonization with NFGNB [1]. Nevertheless, different bacterial species in CF-patients display distinct degrees of pathogenicity, thus requiring different clinical management [2]. Correct identification of these bacteria by conventional microbiology methods is often limited due to low biochemical reactivity [3]. Moreover, isolates from patients with chronic pathogenic colonization often lose their characteristic phenotypes or growth conditions [4]. In addition, bacterial misidentification could be due to the fact that the species are not in the database of the commercial systems used in clinical laboratories. In these cases, molecular tools such as 16S rDNA gene

sequencing provide reliable results, although it might have problems to assign at specie level [5].

Recently, several studies have reported the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for bacterial identification, including NFGNB [6, 7]. This technique has been recently introduced in clinical microbiology laboratories [8]. It is based on relative molecular masses of proteins producing specific mass-spectral fingerprints for different organisms. The reproducibility of the MALDI-TOF MS is based on the measurement of high-abundance proteins, including ribosomal proteins and the final identification is not significantly influenced by variability in environmental or growth conditions.

In this study we reassessed by MALDI-TOF MS historical routine identification results obtained by conventional methods of NFGNB recovered from sputum samples from 70 CF-patients attended in our CF-Unit from 1994 to 2009 and obtained during routine follow-up visits or during exacerbations. *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* isolates were

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excluded from our study as they can be normally identified by routine conventional methods [9, 10].

A total of 182 isolates (66 *Achromobacter* spp., 54 *Burkholderia* spp., 16 *Acinetobacter* spp., 7 non-aeruginosa *Pseudomonas*, 8 *Chryseobacterium* spp., 7 *Bordetella* spp., 4 *Ralstonia* spp., 4 *Ochrobactrum anthropi*, and 16 other NFGNB) sequentially isolated during the study period and stored at -80°C were studied. Conventional routine identification at the species level was performed at the time of isolation over the 15 year period using various phenotypic tests including the PASCO (Difco, Detroit, MI) or WIDER (Fco. Soria-Melguizo, Madrid, Spain) systems. When this identification did not satisfy the criteria for acceptance, then the API20NE (BioMérieux, Marcy-L'Étoile, France) gallery was performed. The identification using the MALDI-TOF MS was performed by the direct colony method with a Microflex LT instrument using the FlexControl 3.0 and MALDI BioTyper 2.0 software (Bruker Daltonics GmbH, Leipzig, Germany). Only scores above 1.7 were considered for identification and names of the best match of the database were recorded. When MALDI-TOF MS and phenotypic identifications agreed, no further methods were performed. Discrepancies at genus level were resolved with a sequenced-based molecular technique (PCR of 16S rDNA) that was considered as the “gold standard” identification method [5]. Moreover, this molecular technique was also applied when MALDI-TOF MS yield no identification. No further molecular characterization techniques (e.g. *recA* or *gyrB*-based sequencing) were used to improve isolate identification at species level.

Conventional and MALDI-TOF MS identification results are shown in Table 1. Performance of the MALDI-TOF MS system was significantly better for identification than conventional routine biochemical phenotypic tests. Overall, one hundred seventy one out of 182 isolates (94%) were identified by mass spectrometry. Eighty percent (145/182) of studied isolates displayed identical identification at genus level when compared phenotypic and MALDI-TOF MS methods but decreased to 68% at species level when compared with routine identification obtained with conventional methods (see below).

Among previously identified *Achromobacter xylosoxidans* isolates by conventional methods (n=66), only fifty-six (85%) confirmed this identification by MALDI-TOF MS at specie level whereas the other isolates were identified as different *Achromobacter* species, and also to some emerging pathogens in CF, including *Pandora* spp. This result is of clinical interest as *A. xylosoxidans* within *Achromobacter* genera has been demonstrated to cause persistent respiratory tract infection and inflammation in CF-patients similar to that of *P. aeruginosa* [11]. Similarly, MALDI-TOF MS is able to differentiate different species within the *Burkholderia cepacia* complex [6, 7]. In our study, we speciated *B. cepacia* isolates by MALDI-TOF MS as *Burkholderia cenocepacia* (n=29), *Burkholderia vietnamiensis* (n=8), *Burkholderia cepacia* (n=2) and *Burkholderia multivorans* (n=2). Speciation within this complex is specifically recommended not only for the clinical management of these patients but also for infection control measures [12, 13]. Conversely, seven isolates in our study previously identified as

B. cepacia were speciated by MALDI-TOF MS as *Burkholderia gladioli*, showing that conventional identification was not reliable for this specie (Table 1). MALDI-TOF MS spectra from our *B. gladioli* isolates accurately matched with that obtained with a quality control *B. gladioli* strain (data not shown).

In addition, six isolates were confirmed as non belonging to the *B. cepacia* complex. These included three isolates of *Pandora* spp. which had never been previously identified by conventional methods in our laboratory. This organism has been recovered from the respiratory tracts of CF-patients and differentiation from *B. cepacia* complex organisms may be problematic [14].

In the case of other organisms rarely isolated from CF-patients included in our study, we demonstrated that conventional identification methods were also misleading and unreliable due to the absence of different species in the manufacturers' databases. This was the case of different species within the *Acinetobacter* [15] and *Chryseobacterium* [16] genus. Also, MALDI-TOF MS improved the identification of five strains previously characterized as *Pseudomonas fluorescens* group. On the contrary, an accurate identification of *Ralstonia pickettii* (n=4) by automated phenotypic identification methods was obtained.

Discrepancies (n=19) of routine phenotypic and MALDI-TOF MS identifications at genus level were resolved by 16S rDNA gene sequencing in favor of the last one. Lack of identification (6% of isolates, n=11) with MALDI-TOF MS was resolved by 16S rDNA gene sequencing as *Ralstonia* spp. (n=5), *Bordetella petrii* (n=3), *Chryseobacterium* spp. (n=2) and *Sphingobacterium spiritovorum* (n=1), all of them occasionally isolated from CF-patients [16] (Table 1). “No identification” results by MALDI-TOF MS were clearly associated with an absence of sufficient spectra from suitable reference strains in the database.

Previous studies described the performance of MALDI-TOF MS for bacterial identification [17]. Our current manuscript shows how this system, using a commercial database, improves routine identification of CF isolates. We showed that MALDI-TOF MS is a reliable and versatile tool for identification of NFGNB bacteria recovered from CF-patients that usually have limited biochemical reactivity leading to misidentification when using classical phenotypic approaches. Despite its accuracy, MALDI-TOF MS database needs to be updated and enlarged with a wider range of microbial species including infrequent or rare organisms recovered from CF-patients. The implementation of MALDI-TOF MS system in the routine identification processes of CF isolates will not only reduce time-to-report but also provide value information for antimicrobial treatment. Moreover, correct identification of these organisms has been highlighted in the infection control guidelines of CF-patients as a first step in their clinical management [18, 19].

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Table 1
Comparative identification of non-fermenting Gram-negative bacilli by routine methods and MALDI TOF MS.

Phenotypic identification (no.)	MALDI-TOF MS identification (no.)	Resolution of discrepant results using 16S rDNA gene sequencing (no.)
<i>Achromobacter xylosoxidans</i> (66)	<i>Achromobacter xylosoxidans</i> (56)	
	<i>Achromobacter ruhlandii</i> (1)	
	<i>Achromobacter insolitus</i> (1)	
	<i>Achromobacter spanius</i> (2)	
	<i>Pandoraea</i> spp. (3)	<i>Pandoraea</i> spp. (3)
	<i>Acinetobacter</i> spp. (1)	<i>Acinetobacter</i> spp. (1)
<i>Burkholderia cepacia</i> complex (54)	No identification (2)	<i>Ralstonia</i> spp. (2)
	<i>Burkholderia cenocepacia</i> (29)	
	<i>Burkholderia vietnamiensis</i> (8)	
	<i>Burkholderia cepacia</i> (2)	
	<i>Burkholderia multivorans</i> (2)	
	<i>Burkholderia gladioli</i> (7)	
<i>Acinetobacter lwoffii</i> complex (8)	<i>Pandoraea</i> spp. (3)	<i>Pandoraea</i> spp.(3)
	<i>Achromobacter xylosoxidans</i> (2)	<i>Achromobacter xylosoxidans</i> (2)
	<i>Pseudomonas huttiensis</i> (1)	<i>Pseudomonas huttiensis</i> (1)
	<i>Acinetobacter ursingii</i> (3)	
	<i>Acinetobacter johnsonii</i> (1)	
	<i>Acinetobacter</i> spp. (3)	
<i>Acinetobacter baumannii</i> (8)	<i>Agrobacterium tumefaciens</i> (1)	<i>Agrobacterium tumefaciens</i> (1)
	<i>Acinetobacter baumannii</i> (1)	
	<i>Acinetobacter</i> spp. (3)	
	<i>Ochrobactrum anthropi</i> (1)	<i>Ochrobactrum</i> spp. (1)
	<i>Pseudomonas</i> spp. (1)	<i>Pseudomonas</i> spp. (1)
	<i>Achromobacter</i> spp. (1)	<i>Achromobacter</i> spp. (1)
<i>Pseudomonas fluorescens/putida</i> (6)	No identification (1)	<i>Bordetella petrii</i> (1)
	<i>Pseudomonas libanensis</i> (2)	
	<i>Pseudomonas gessardi</i> (1)	
	<i>Pseudomonas tolaasii</i> (1)	
	<i>Pseudomonas koreensis</i> (1)	
	<i>Achromobacter xylosoxidans</i> (1)	<i>Achromobacter xylosoxidans</i> (1)
<i>Pseudomonas stutzeri</i> (1)	<i>Pseudomonas stutzeri</i> (1)	
<i>Chryseobacterium indologenes</i> (6)	<i>Chryseobacterium indologenes</i> (3)	
	<i>Chryseobacterium joostei</i> (3)	
	<i>Brevibacillus reuszeri</i> (1)	<i>Brevibacillus reuszeri</i> (1)
<i>Chryseobacterium meningosepticum</i> (2)	No identification (1)	<i>Chryseobacterium gleum</i> (1)
	<i>Bordetella bronchiseptica</i> (4)	
<i>Bordetella bronchiseptica</i> (7)	No identification (3)	<i>Bordetella petrii</i> (2), <i>Ralstonia</i> spp. (1)
	<i>Ralstonia pickettii</i> (4)	
	<i>Ochrobactrum anthropi</i> (2)	
<i>Ralstonia pickettii</i> (4)	<i>Ochrobactrum anthropi</i> (2)	
	<i>Ochrobactrum grignonense</i> (1)	
	No identification (1)	<i>Chryseobacterium</i> spp. (1)
<i>Agrobacterium tumefaciens</i> (1)	<i>Agrobacterium tumefaciens</i> (1)	
	<i>Alcaligenes</i> spp. (1)	
<i>Chromobacterium violaceum</i> (1)	<i>Achromobacter insolitus</i> (1)	
	<i>Pseudomonas fragi</i> (1)	<i>Pseudomonas</i> spp. (1)
<i>Delftia acidovorans</i> (1)	<i>Delftia acidovorans</i> (1)	
	<i>Acinetobacter junii</i> (1)	<i>Acinetobacter johnsonii</i> (1)
<i>Empedobacter brevis</i> (1)	<i>Arthrobacter castelli</i> (1)	<i>Arthrobacter</i> spp. (1)
	<i>Brevundimonas vesicularis</i> (1)	<i>Brevundimonas diminuta</i> (1)
NFGNB (11)	<i>Bordetella bronchiseptica</i> (2)	<i>Bordetella</i> spp. (2)
	<i>Chryseobacterium indologenes</i> (1)	<i>Chryseobacterium</i> spp. (1)
	<i>Oligella urethralis</i> (1)	<i>Oligella</i> spp. (1)
	<i>Pandoraea</i> spp. (1)	<i>Pandoraea</i> spp. (1)
	<i>Sphingobacterium spiritivorum</i> (1)	<i>Sphingobacterium spiritivorum</i> (1)
	No identification (3)	<i>Ralstonia</i> spp.(2), <i>Chryseobacterium</i> spp.(1)

NFGNB: non fermenting Gram-negative bacilli.

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