European Journal of Clinical Microbiology & Infectious Diseases (2020) 39:559–567
https://doi.org/10.1007/s10096-019-03758-x

ORIGINAL ARTICLE

Assessment of VITEK® MS IVD database V3.0 for identification of *Nocardia* spp. using two culture media and comparing direct smear and protein extraction procedures

T. Durand[1] · F. Vautrin[2] · E. Bergeron[2] · V. Girard[3] · S. Polsinelli[3] · V. Monnin[3] · G. Durand[3] · O. Dauwalder[1] · O. Dumitrescu[1] · F. Laurent[1] · V. Rodríguez-Nava[1,2]

Received: 30 October 2019 / Accepted: 30 October 2019 / Published online: 22 November 2019

Abstract

We assessed the performance of the VITEK® MS IVD V3.0 matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-ToF MS) V3.0 database for the identification of *Nocardia* spp. as compared with targeted DNA sequencing. A collection of 222 DNA sequence-defined *Nocardia* spp. strains encompassing 18 different species present or not in the database was tested. Bromocresol purple agar (BCP) and Columbia agar +5% sheep's blood (COS) culture media were used together with two different preparation steps: direct smear and a "3 attempts" procedure that covered (1) spotting of an extract, (2) new spotting of the same extract, and (3) spotting of a new extract. The direct smear protocol yielded low correct identification rates (≤ 15% for both media) whereas protein extraction yielded correct identification results (> 67% regardless of the media used.). The use of 2 additional attempts using repeat or new extracts increased correct identification rates to 87% and 91% for BCP and COS, respectively. When using the 3 attempts procedure, the best identification results, independent of media types, were obtained for *N. farcinica* and *N. cyriacigeorgica* (100%). Identification attempts 2 and 3 allowed to increase the number of correct identifications (BCP, +20%; COS, +13%). The enhancement in performance during attempts 2 and 3 was remarkable for *N. abscessus* (81% for both media) and low prevalence species (BCP, 70%; COS, 85%). Up to 3.4% and 2.4% of the strains belonging to species present in the database were misidentified with BCP and COS media, respectively. In 1.9% of the cases for BCP and 1.4% for COS, these misidentifications concerned a species belonging to the same phylogenetic complex. Concerning strains that are not claimed in the V3.0 database, *N. puris* and *N. goodfellowi* generated "No identification" results and 100% of the strains belonging to *N. arthritidis*, *N. cerradoensis*, and *N. altamirensis* yielded a misidentification within the same phylogenetic complex. Vitek® MS IVD V3.0 is an accurate and useful tool for identification of *Nocardia* spp.

Keywords *Nocardia* spp. · MALDI-ToF · BCP · COS · DNA sequencing

Introduction

Nocardia species are filamentous, Gram-positive bacteria belonging to the order *Corynebacteriales*. More than 100 *Nocardia* species have been characterized, among which approximately half are of medical importance [1]. Members of this genus are cosmopolitan and ubiquitous in the environment. Nocardiosis is primarily opportunistic and affects immunocompromised patients mostly [2, 3], although immunocompetent patients can also be affected [4]. Although cutaneous and soft tissue infections predominate, the most common presentation is pulmonary nocardiosis [5–7]. The mortality associated with these infections remains high [8], which underlines the need for rapid and effective treatment. Intrinsic antimicrobial susceptibility patterns differ between species [9] and render rapid identification of the species essential. Currently, *Nocardia* spp. identification is based on 16S rRNA, *hsp65*, *secA1*, *rpoB*, and *gyrB* gene sequencing [10–12]. Although these techniques are specific and sensitive,

✉ V. Rodríguez-Nava
veronica.rodriguez-nava@univ-lyon1.fr

[1] Institut des Agents infectieux, Centre de Biologie et Pathologies Nord, Hôpital de la Croix Rousse, Lyon, France

[2] UMR CNRS 5557, Ecologie Microbienne – Groupe de Recherche "Pathogènes Opportunistes et Environnement" – ISPB-Faculté de Pharmacie, Université Lyon 1, Lyon, France

[3] bioMérieux France, Microbiology R&D, La Balme-les-Grottes, France

their drawbacks include costs, duration, and limited availability. Thus, samples must often be transported, delaying the identification of the pathogen. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS), a tool that is now widely used to identify common bacterial and yeast species, is a promising technology for *Nocardia* spp. identification. It is easy to use, fast, and cost-effective and, hence, an interesting alternative to molecular methods. The Bruker BioTyper system has been evaluated for clinical *Nocardia* spp. identification [11, 13–15] as was the VITEK® MS IVD system [16–18].

Our objective was to evaluate the performance of the VITEK® MS IVD V3.0 database for the identification of *Nocardia* spp. strains and different strategies for the specimen preparation step were assessed. In parallel, the performance of bromocresol purple agar (BCP) was compared with those obtained on Columbia agar +5% sheep's blood (COS).

Materials and methods

Bacterial strains

The collection of isolates used in this study was from the Observatoire Français des Nocardioses (OFN), Lyon, France. This collection is composed of 222 strains with 131 isolates specifically collected in 2014 from the OFN, Institut des Agents Infectieux (French epidemiology, [19]). In addition, for each species tested, we have included the corresponding type strain (Table 1). All isolates were previously identified at species level by sequencing the 16S rRNA gene [20], and DNA sequencing was performed by Biofidal (Vaulx-en-Velin, France). When identification was not possible, a 441-bp fragment of the *hsp65* gene was amplified and sequenced [20]. The sequences were analyzed by BLAST (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) following the identification criteria of the Clinical and Laboratory Standards Institute (CLSI, [21]). Isolates were stored at − 80 °C and were subcultured on two different media (bromocresol purple agar (BCP, bioMérieux Ref. 43021) and Columbia agar +5% sheep's blood (COS, bioMérieux Ref. 43041) at 37 °C for 72 h. BCP is a poor culture medium not used to build the MS database but successfully used in OFN as it shows rapid growth for all *Nocardia* spp. In contrast, COS is a rich culture medium which has been used to build MS database and is recommended by the manufacturer.

Protein extraction

All isolates were extracted according to the bioMérieux recommendations using the VITEK® MS IVD *Mycobacterium*/*Nocardia* kit (bioMérieux Ref. 415659). A 1 μL loop full of organisms was transferred into a 1.5mL Eppendorf tube containing 500 μL of 70% ethanol and approximately 200 μL of 0.5-mm glass beads. The mixture was vortexed for 15 min with a Genie 2 Vortex with a 13000-V1-24 Vortex adaptor (MoBio, Qiagen) and then was incubated at room temperature for 10 min. The suspension was briefly vortexed and then transferred into an empty 1.5mL Eppendorf tube (avoiding the transfer of any glass beads) and centrifuged for 2 min at 14,000 rpm. The ethanol supernatant was removed, and the pellet was re-suspended in 10 μL of 70% formic acid. The tube was briefly vortexed, 10 μL of acetonitrile was added, and the tube was vortexed again and then centrifuged for 2 min at 14,000 rpm. The resulting supernatant was used for analysis by MALDI-ToF MS.

Sample deposits

For the two media types, the isolates were deposited in two different ways: (i) a direct smear by which a loop full of bacteria was directly applied as a thin film on a spot of a target slide (disposable 48 well stainless steel target slides, bioMérieux Vitek MS), (ii) an extract deposit by which 1 μL of supernatant was deposited on a spot of a target slide as described above. In either case, the deposit was allowed to dry. Next, the deposit was overlaid with 1 μL of α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution and was allowed to dry again. The *Escherichia coli* reference strain ATCC 8739 was used on each plate for instrument calibration according to the manufacturer's instructions. Positive-control organism *N. farcinica* type strain (DSM 43665^T) was spotted on the slide using the protocols described in this study. Finally, the slide was loaded into the VITEK® MS instrument.

Sample analysis

All isolates were analyzed using the manufacturer's recommended settings, and the mass spectra obtained were compared with the V3.0 database. An identification associated with a confidence level was produced by the Myla software.

Identification procedures

As seen above, two different preparation steps were tested. The results of each method were compared and the one yielding to better correct identification rates underwent a several attempts procedure in order to succeed in identifying strains for which a "no identification" result was obtained. In the case direct smear yielded the best results, 2 new deposits were foreseen. For preparation steps based on protein extraction, two new attempts were planned as follows: (i) new spotting of the same extract previously defrosted, and (ii) spotting of a new extract (Fig. 1).

Table 1 Species and number of strains for each one, used in this study. Prevalence classification is done according to Lebeaux et al. (19)

Species	Number of strains including type strain	Type strain code
High prevalence	110	
N. farcinica	43	DSM 43665 ^{T}
N. nova	26	DSM 43256 ^{T}
N. abscessus	21	DSM 44432 ^{T}
N. cyriacigeorgica	19	DSM 44484 ^{T}
Intermediate prevalence	55	
N. wallacei	16	DSM 45136 ^{T}
N. veterana	14	DSM 44445 ^{T}
N. otitidiscaviarum	13	DSM 43242 ^{T}
N. brasiliensis	11	DSM 43758 ^{T}
Low prevalence	40	
N. beijingensis	12	DSM 44636 ^{T}
N. paucivorans	11	DSM 44386 ^{T}
N. pseudobrasiliensis	10	DSM 44290 ^{T}
N. neocaledoniensis	3	DSM 44717 ^{T}
N. asteroides	4	DSM 43757 ^{T}
Sub-total	203	
*N. cerradoensis**	3	DSM 44546 ^{T}
*N. altamirensis**	2	DSM 44997 ^{T}
*N. puris**	6	DSM 44599 ^{T}
*N. goodfellowi**	3	DSM 45516 ^{T}
*N. arthritidis**	5	DSM 44731 ^{T}
Total	222	

*Low prevalence species that are not present in the V3.0 database

Identification criteria

The result was considered correct at the species level if a single species identification associated with a confidence level > 99% was obtained and matched the identification obtained by the reference method (16S rRNA/*hsp65* sequencing). The identification was considered correct at complex level if the system yielded a slash line result (i.e. *Species 1/Species 2*) suggesting two *Nocardia* species, one matching with the one obtained by the reference method and if the other one belonged to the same phylogenetic complex according to McTaggart et al. [12] .

Results

Performance for species present in the VITEK MS V3.0 database

Out of 222 tested strains, 203 belonged to species present in the VITEK MS V3.0 database and were first submitted to a standard smear based identification process. The system yielded poor identification rates of 15% and 11% for BCP and COS media, respectively. The protein extraction procedure allowed better results as the system yielded correct identification for 67% of isolates from the BCP medium and 78% from the COS medium. Taking into account these results, the preparation step based on protein extraction was the one that underwent two more attempts (herein, "attempt 2" and "attempt 3") in order to increase correct identification rates.

Spotting of the same extract after defrosting (attempt 2) for previously unidentified isolates (68 for BCP and 45 for COS media) allowed to increase correct identification rates, 24/68 (35%) for BCP medium and 15/45 (33%) for COS medium (Table 2). Spotting of a new extract (attempt 3) for unidentified isolates in attempt 2 (44 strains for BCP and 30 for COS media) further helped to increase correct identification rates, 18/44 strains (41%) for BCP medium and 11/30 strains (37%) for COS medium. So, thanks to the second and third attempts, more than a half of the strains not identified in the first attempt were identified as follows: 42/68 (62%) with BCP medium and 26/45 (58%) with COS. Overall, we reached correct identification at species or complex level at 87% (an increment of 20%) for the BCP medium and at 91% (an increment of 13%) for the COS medium (Table 2).

For the most prevalent species (*N. farcinica*, *N. nova*, *N. abscessus*, *N. cyriacigeorgica*), which account for 54% of all tested strains, high identification rates were obtained: up to 93% (101/109) and 94% (102/109) for BCP and COS agar, respectively. Again, attempts 2 and 3 lead to a considerable increase of correct identification rates regarding those of

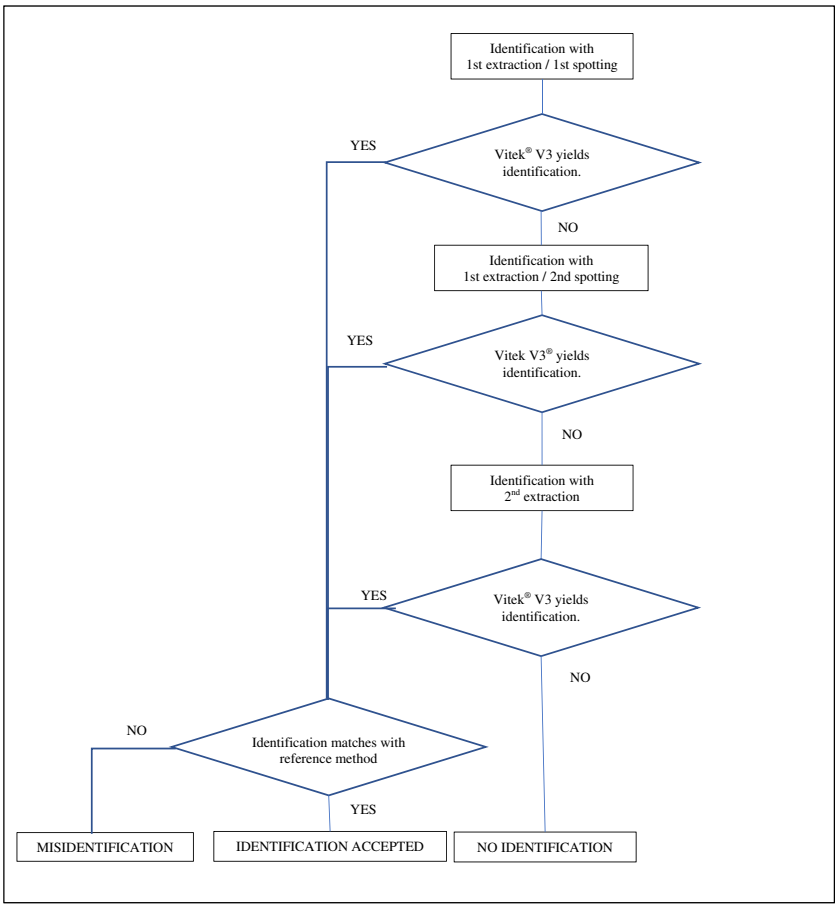

Fig. 1 Decision algorithm

attempt 1 (+21% for BCP and +10% for COS). *N. farcinica* and *N. cyriacigeorgica* were 100% correctly identified, mostly in the first attempt. Regarding *N. nova*, more than 80% of the strains were identified at complex level and displayed as a slash line "*N. nova 50%/N. africana 50%*". For this species and particularly on BCP medium, the last two attempts increased the number of correct identifications by 23% and for COS medium almost all the correct identifications (85%) were obtained upon first spotting. Regarding the *N. abscessus* strains, Vitek® MS IVD V3.0 yielded a correct identification from the first spot in half of the cases. Attempts 2 and 3 allowed to increase the overall correct identification rates (BCP +29%, COS +24%).

Concerning the species with intermediate prevalence (*N. wallacei*, *N. brasiliensis*, *N. veterana*, *N. otitidiscaviarum*), attempts 2 and 3, allowed to increase the correct identification rates (+15% for both media) for reaching a high cumulative identification rate of 89% (48/54) of isolates for both media. However, there were slightly lower correct identification rates for *N. veterana* compared with the 3 other species of this group (Table 2).

For species with low prevalence (*N. paucivorans*, *N. pseudobrasiliensis*, *N. asteroides sensu stricto*, *N. beijingensis*, *N. neocaledoniensis*), the cumulative identification rates were satisfactory. Up to 70% (28/40) of isolates were identified using the BCP medium and 85% (34/40) with the COS medium. For reaching these values, attempts 2 and 3 were helpful (BCP +27%, COS +20%). The full identification procedure for *N. paucivorans* and *N. pseudobrasiliensis* allowed to identify 82% (9/11) and 70% (7/10) of the strains for BCP medium and 100% of the strains for COS medium. For *N. beijingensis*, only 67% (8/12) with the "3 attempts" procedure of isolates were correctly identified with both media. The 3 isolates of *N. neocaledoniensis* were correctly identified regardless of the medium. Regarding the 4 strains of *N. asteroides stricto sensu*, only one of them was correctly identified at species level using both media. In addition, a correct identification at complex level under the form "*N. asteroides/N. neocaledoniensis*" was obtained only for one strain for COS medium.

Regarding misidentification of strains belonging to species present in the manufacturer's database, 3.4% of the isolates were misidentified with BCP (7/203) and 2.4% with COS (5/203) media types (Table 3). One strain of *N. nova* was identified as *N. veterana* with both media. One strain of *N. abscessus* was identified as *N. veterana* and another one was identified as *N. beijingensis* with both media. One strain of *N. veterana* was identified as *N. cyriacigeorgica* with both media, too. One strain of *N. pseudobrasiliensis* was identified

Table 2 Number of correctly identified isolates from the subset of species present in the V3.0 database ("% initial" stands for the percentage of correct identifications regarding the initial number of strains; "% attempt" stands for the percentage of correct identifications regarding the number of strains used exclusively in this attempt)

Attempt 1: spotting of an extract				Attempt 2: new deposit of the same extract			Attempt 3: deposit of a new extract			Cumulative total	
Species	Initial no. of isolates	BCP (% initial)	COS (% initial)	No. of isolates (BCP/COS)	BCP (% attempt) (% initial)	COS (% attempt) (% initial)	No. of isolates (BCP/COS)	BCP (% attempt) (% initial)	COS (% attempt) (% initial)	BCP (% initial)	COS (% initial)
High prevalence	109	78 (72)	92 (84)	31/17	14 (45)(13)	6 (35)(6)	17/11	9 (53)(8)	4 (36)(4)	101 (93)	102(94)
N. farcinica	43	33 (77)	41 (95)	10/2	8 (80)(19)	1 (50)(2)	2/1	2 (100)(5)	1 (100)(2)	43 (100)	43 (100)
N. nova	26	16 (62)	22 (85)	10/4	3 (30)(12)	1 (25)(4)	7/3	3 (43)(12)	0 (0)(0)	22 (85)[1]	23 (88)[1]
N. abscessus	21	11 (52)	12 (57)	10/9	3 (30)(14)	3 (33)(14)	7/6	3 (43)(14)	2 (33)(10)	17 (81)	17 (81)
N. cyriacigeorgica	19	18 (95)	17 (89)	1/2	0 (0)(0)	1 (50)(5)	1/1	1 (100)(5)	1 (100)(5)	19 (100)	19 (100)
Intermediate prevalence	54	40 (74)	40 (74)	14/14	5 (36)(9)	4 (29)(7)	9/10	3 (33)(6)	4 (40)(7)	48 (89)	48 (89)
N. wallacei	16	12 (75)	10 (63)	4/6	2 (50)(13)	2 (33)(13)	2/4	1 (50)(6)	3 (75)(19)	15 (94)	15 (94)
N. veterana	14	11 (79)	10 (71)	3/4	0 (0)(0)	1 (25)(7)	3/3	0 (0)(0)	0 (0)(0)	11 (79)	11 (79)
N. otitidiscaviarum	13	8 (62)	11 (85)	5/2	2 (40)(15)	0 (0)(0)	3/2	2 (67)(15)	1 (50)(8)	12 (92)	12 (92)
N. brasiliensis	11	9 (82)	9 (82)	2/2	1 (50)(9)	1 (50)(9)	1/1	0 (0)(0)	0 (0)(0)	10 (91)	10 (91)
Low prevalence	40	17 (43)	26 (65)	23/14	5 (22)(13)	5 (36)(13)	18/9	6 (33)(15)	3 (33)(8)	28 (70)	34 (85)
N. beijingensis	12	5 (42)	6 (50)	7/6	2 (29)(17)	1 (17)(8)	5/5	1 (20)(8)	1 (20)(8)	8 (67)	8 (67)
N. paucivorans	11	4 (36)	9 (82)	7/2	2 (29)(18)	2 (100)(18)	5/0	3 (60)(27)	-	9 (82)	11 (100)
N. pseudobrasiliensis	10	5 (50)	6 (60)	5/4	0 (0)(0)	2 (50)(20)	5/2	2 (40)(20)	2 (100)(20)	7 (70)	10 (100)
N. neocaledoniensis	3	2 (67)	3 (100)	1/0	1 (100)(33)	-	0/0	-	-	3 (100)	3 (100)
N. asteroides	4	1 (25)	2 (50)	3/2	0 (0)(0)	0 (0)(0)	3/2	0 (0)(0)	0 (0)(0)	1 (25)	2 (50)[2]
Total	**203**	**135 (67)**	**158 (78)**	**68/45**	**24 (35)(12)**	**15 (33)(7)**	**44/30**	**18 (41)(9)**	**11 (37)(5)**	**177 (87)**	**184 (91)**

[1] *N. nova* was identified using slash line *N. nova*/*N. africana*

[2] One *N. asteroides* strain was identified using slash line *N. asteroides*/*N. neocaledoniensis*

as *Pseudomonas oryzihabitans* with BCP medium but a correct identification with COS medium was obtained. A re-extraction of the strain yielded the correct identification, so contamination is plausible, which in the end was not possible to verify. One strain of *N. asteroides sensu stricto* was identified as *N. neocaledoniensis* with BCP medium. However, a correct identification at complex level was obtained with COS medium and a second strain of *N. asteroides sensu stricto* was identified as *N. neocaledoniensis* with both media.

Performance for species absent from the VITEK MS V3.0 database

Regarding the 19 strains belonging to 5 species absent from the V3.0 database (*N. altamirensis*, *N. arthritidis*, *N. cerradoensis*, *N. goodfellowi*, *N. puris*), they were either not identified or misidentified. All the strains of *N. goodfellowi* and *N. puris* (including their type strains) yielded no identification. For the remaining 10 strains, misidentifications were obtained. Up to 4/5 isolates of *N. arthritidis* were identified as *N. abscessus*. One isolate was identified as *N. beijingensis* with BCP but no identification could be obtained with COS. The 3 isolates of *N. cerradoensis* were identified as *N. nova* 50%/*N. africana* 50%, and both strains of *N. altamirensis* were identified as *N. brasiliensis*.

Discussion

In this study, the direct smear preparation step was evaluated for the first time with the Vitek® MS IVD and was found not satisfactory. We demonstrated in this study that for VITEK® MS IVD V3.0, an extraction is needed to obtain good identification rates for *Nocardia* spp. New attempts were needed as 67% and 78% of the strains were identified during the first spotting of the first extract with BCP and COS media, respectively. The necessity of repeating identification procedure by different means with this system has also been observed by Body et al. [18] who needed to repeat identification procedures for 33% of their *Nocardia* spp. strains. This study shows that the "3 attempts" procedure with both media lead to final identification rates (BCP 87%, COS 91%) which match with those of Body et al (90%) [18].

Different preparation steps are referred in the literature for other MALDI-ToF MS-based systems that may be worth testing with VITEK® MS IVD V3.0. For example, for Microflex LT, some recent studies suggest a halfway technique between direct smear and extraction: the direct on-target extraction [22]. Further studies should be done for VITEK® MS IVD V3.0 to assess identification accuracy when using this kind of a more rapid preparation step.

The impact of the culture medium and incubation time on the quality of the spectra has already been discussed [18, 23, 24, 25]; however, conclusions are contradictory. Khot et al. [24] and McTaggart et al. [25] show that the incubation time impacts the quality of the spectra since better results were obtained with a short incubation time. Moreover, McTaggart et al. [25] concluded that the type of culture medium used has an indirect impact since rich media, such as COS, allow faster and more abundant growth which can result in spectra of better quality. However, according to Body et al. [18], identification results can be identical independently of the culture medium but their study was limited to media used for the building of the database.

Our results agree with McTaggart et al. [25] as we observed slightly better correct identification rates with a rich medium like COS (91%) compared with a poor one like BCP (87%) which nevertheless gave good results. However, we demonstrate the possibility to use a medium like BCP for identification purpose even if it has not been used to build the database. Cumulative results are indeed comparable with those of COS medium and especially for highly and intermediate prevalence species.

For *N. nova* strains for which VITEK® MS IVD V3.0 yielded a correct identification (85% BCP; 88% COS), only a complex level result displayed as "*N. nova* 50% / *N. africana* 50%" could be obtained. As explained by Girard et al. [16], the *N. nova* and *N. africana* species are currently indistinguishable by the VITEK® MS IVD V3.0 and are therefore only identified at the complex level. In fact, the taxonomy of the genus *Nocardia* spp. has evolved considerably in recent years and in addition to *N. nova* and *N. africana*, more species have been added to the *N. nova* complex including *N. veterana*, *N. cerradoensis*, *N. kruczakiae*, *N. aobensis*, *N. mikamii*, and *N. elegans* [12, 26]. Hence, some of the observed misidentifications (1 out of the 26 tested strains of *N. nova* was identified as *N. veterana* and all the 3 strains of *N. cerradoensis* as *N. nova/N. africana*) remain understandable. In a similar way, the *N. abscessus* is very close to other species such as *N. beijingensis*, *N. arthritidis*, and *N. asiatica* forming a phylogenetic clade [1, 12, 26]. On the 7 misidentifications obtained for those species, 6 of them were obtained within the complex. For example, *N. arthritidis* strains were misidentified as *N. abscessus* or *N. beijingensis*. Even if *N. arthritidis* is not present in the database, the result yielded by VITEK® MS IVD V.3.0 remained inside the correct phylogenetic complex. Body et al. [18] observed several similar misidentifications.

Some other misidentifications yielded by the system are also understandable. For example, *N. altamirensis* is misidentified as *N. brasiliensis*. These two species belong to the same phylogenetic clade which also encompasses *N. boironii* and *N. vulneris* [27]. In the same way, we observed a misidentification of a *N. asteroides* strain as

Table 3 Misidentifications obtained with Vitek® MS IVD V3.0 and two different media for tested *Nocardia* strains compared with the identification at species level obtained with the reference method. *BCP*, bromocresol purple agar; *COS*, Columbia agar +5% sheep's blood

		Identification by the Vitek® MS IVD	
		Medium	
Species[1]	Initial number of strains for this species	BCP	COS
N. nova	26	*N. veterana*	*N. veterana*
N. abscessus	21	*N. veterana*	*N. veterana*
N. abscessus		*N. beijingensis*	*N. beijingensis*
N. veterana	14	*N. cyriacigeorgica*	*N. cyriacigeorgica*
N. pseudobrasiliensis	10	*Pseudomonas oryzihabitans*	*Correct Id*
N. asteroides	4	*N. neocaledoniensis*	*Correct Id (complex: N. asteroides/N. neocaledoniensis)*
N. asteroides		*N. neocaledoniensis*	*N. neocaledoniensis*
*N. arthritidis**	5	*N. abscessus*	*N. abscessus*
*N. arthritidis**		*N. abscessus*	*N. abscessus*
*N. arthritidis**		*N. abscessus*	*N. abscessus*
*N. arthritidis**		*N. beijingensis*	*No Id*
N. arthritidis ^{T}*		*N. abscessus*	*N. abscessus*
*N. cerradoensis**	3	*N. nova 50%/N. africana 50%*	*N. nova 50%/N. africana 50%*
*N. cerradoensis**		*N. nova 50%/N. africana 50%*	*N. nova 50%/N. africana 50%*
N. cerradoensis ^{T} *		*N. nova 50%/N. africana 50%*	*N. nova 50%/N. africana 50%*
*N. altamirensis**	2	*N. brasiliensis*	*N. brasiliensis*
N. altamirensis ^{T}*		*N. brasiliensis*	*N. brasiliensis*

[1] Species identification based in reference method

*Species that are not present in the V3.0 database

N. neocaledoniensis, which is clustered in the same phylogenetic complex. This misidentification was also observed by Body et al. [18] for 3/19 of their *N. asteroides* isolates. Misidentifications regarding low prevalence species can be related to the availability of low numbers of spectra for these species [17].

Nowadays, MALDI ToF MS seems not to have sufficient discriminatory power to distinguish all species belonging to the different phylogenetic clades. Some species misidentifications are problematic as such species do not always present the same antibiotic profiles, potentially leading to inappropriate patient handling. This is especially true for the *N. abscessus* complex, since *N. beijingensis* and *N. asiatica* are usually susceptible to imipenem ([28] and personal data) in contrast to *N. abscessus* and *N. arthritidis* ([29] and personal data) which are generally resistant. This divergence of antibiotic profiles can also be observed inside the *N. brasiliensis* complex. For the *N. nova* complex, misidentifications have a lesser clinical impact as the species in this complex show similar antibiotic profiles but the prevalence of these species are different and this can lead to wrongly inferred epidemiological scenarios. In the case of *N. asteroides* and *N. neocaledoniensis*, an accurate identification at species level is not essential, as they are species rarely found in clinical specimens and their susceptibility patterns are not clearly defined.

We suggest that when a species belonging to *N. nova*, *N. abscessus, N. brasiliensis*, or *N. asteroides* complexes is detected, VITEK® MS IVD V3.0 results in identification at the complex level only. In order to avoid therapeutic errors, this kind of result should lead to thorough antibiotic susceptibility testing to help choose appropriate treatment.

Some limitations must be taken into account. Regarding the methodology, new spotting of the same extract was not done immediately after the first spotting as the extract was meanwhile frozen. This can be considered a deviation in

routine laboratory procedure. It is possible that freezing may cause weakening of the bacterial cell walls. Also, the reproducibility of the method was not evaluated. Additional tests are necessary in order to have a better appreciation of the accuracy of these techniques.

VITEK® MS IVD V3.0 yielded good identification rates for *Nocardia* spp. at the species and complex level. Regarding routine processing of *Nocardia* specimens in routine laboratories, extraction gives results above 67% in terms of correct identification rates. In case of "no identification," additional deposit of the same extract or deposit of a new extract can help in obtaining identification rates above 87%. BCP culture medium, which was not used during database development, yields similar identifications as compared with the medium that was used for database development. The best way of avoiding misidentification of low prevalence species is to supplement the database with more strains for these species. Our data show that the VITEK® MS IVD V3.0 can be considered as a useful tool in routine laboratories working with *Nocardia* spp.

Funding information Florian VAUTRIN held a doctoral fellowship from the Region Auvergne-Rhône-Alpes. This study was funded by the VITEK® MS manufacturer (bioMérieux, R&D Microbiologie, La Balme-Les-Grottes, France).

Compliance with ethical standards

Disclaimer The data analysis described here was performed without direct commercial influence by the device manufacturer.

References

1. Conville PS, Brown-Elliott BA, Smith T, Zelazny AM (2017) The complexities of *Nocardia* taxonomy and identification. J Clin Microbiol 56:e01419–e01417. https://doi.org/10.1128/JCM.01419-17
2. Haussaire D, Fournier P-E, Djiguiba K, Moal V, Legris T, Purgus R, Bismuth J, Elharrar X, Reynaud-Gaubert M, Vacher-Coponat H (2017) Nocardiosis in the south of France over a 10-years period, 2004–2014. Int J Infect Dis 57:13–20. https://doi.org/10.1016/j.ijid.2017.01.005
3. Le Coustumier EM, Denes E, Martin C, Weinbreck P (2017) Nocardiose : analyse rétrospective d'une série de 19 cas. Rev Médecine Interne 38:81–89. https://doi.org/10.1016/j.ijid.2017.01.005
4. Wilson JW (2012) Nocardiosis: updates and clinical overview. Mayo Clin Proc 87:403–407. https://doi.org/10.1016/j.mayocp.2011.11.016
5. Anagnostou T, Arvanitis M, Kourkoumpetis TK, Desalermos A, Carneiro HA, Mylonakis E (2014) Nocardiosis of the central nervous system: experience from a general hospital and review of 84 cases from the literature. Medicine (Baltimore) 93:19–32. https://doi.org/10.1097/MD.0000000000000012
6. Jiang Y, Huang A, Fang Q (2016) Disseminated nocardiosis caused by *Nocardia* otitidiscaviarum in an immunocompetent host: a case report and literature review. Exp Ther Med 12:3339–3346. https://doi.org/10.3892/etm.2016.3755
7. Liu WL, Lai CC, Ko WC, Chen YH, Tang HJ, Huang YL, Huang YT, Hsueh PR (2011) Clinical and microbiological characteristics of infections caused by various Nocardia species in Taiwan: a multicenter study from 1998 to 2010. Eur J Clin Microbiol Infect Dis 30:1341–1347. https://doi.org/10.1007/s10096-011-1227-9
8. Munoz J, Mirelis B, Aragon LM, Gutierrez N, Sanchez F, Espanol M, Esparcia O, Gurguí M, Domingo P, Coll P (2007) Clinical and microbiological features of nocardiosis 1997-2003. J Med Microbiol 56:545–550. https://doi.org/10.1099/jmm.0.46774-0
9. Valdezate S, Garrido N, Carrasco G, Medina-Pascual MJ, Villalón P, Navarro AM, Saéz-Nieto JA (2017) Epidemiology and susceptibility to antimicrobial agents of the main *Nocardia* species in Spain. J Antimicrob Chemother 1:754–761. https://doi.org/10.1093/jac/dkw489
10. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ (2006) Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev 19:259–282. https://doi.org/10.1128/CMR.19.2.259-282.2006
11. Xiao M, Pang L, Chen SC-A, Fan X, Zhang L, Li H-X, Hou X, Cheng J-W, Kong F, Zhao Y-P (2016) Accurate identification of common pathogenic *Nocardia* species: evaluation of a multilocus sequence analysis platform and matrix-assisted laser desorption ionization-time of flight mass spectrometry. PLOS ONE 11(1): e0147487. https://doi.org/10.1371/journal.pone.0147487
12. McTaggart LR, Richardson SE, Witkowska M, Zhang SX (2010) Phylogeny and identification of *Nocardia* species on the basis of multilocus sequence analysis. J Clin Microbiol 48:4525–4533. https://doi.org/10.1128/JCM.00883-10
13. Buckwalter SP, Olson SL, Connelly BJ, Lucas BC, Rodning AA, Walchak RC, Deml SM, Wohlfiel SL, Wengenack NL (2016) Evaluation of matrix-assisted laser desorption ionization−time of flight mass spectrometry for identification of mycobacterium species, *Nocardia* species, and other aerobic actinomycetes. J Clin Microbiol 54:376–384. https://doi.org/10.1128/JCM.02128-15
14. Segawa S, Nishimura M, Sogawa K, Tsuchida S, Murata S, Watanabe M, Matsushita K, Kamei K, Nomura F (2015) Identification of *Nocardia* species using matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry. Clin Proteomics 12:6. https://doi.org/10.1186/s12014-015-9078-5
15. Marín M, Ruiz A, Iglesias C, Quiroga L, Cercenado E, Martín-Rabadán P, Bouza E, Rodríguez-Sánchez B (2018) Identification of *Nocardia* species from clinical isolates using MALDI-TOF mass spectrometry. Clin Microbiol Infect 24(12):1342.e5–1342.e8. https://doi.org/10.1016/j.cmi.2018.06.014
16. Girard V, Mailler S, Welker M, Arsac M, Cellière B, Cotte-Pattat P-J, Chatellier S, Durand G, Béni AM, Schrenzel J, Miller E, Dussoulier R, Dunne WM Jr, Butler-Wu S, Saubolle MA, Sussland D, Bell M, van Belkum A, Deol P (2016) Identification of *Mycobacterium* spp. and *Nocardia* spp. from solid and liquid cultures by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). Diagn Microbiol Infect Dis 86:277–283. https://doi.org/10.1016/j.diagmicrobio.2016.07.027
17. Girard V, Mailler S, Polsinelli S, Jacob D, Saccomani MC, Celliere B, Monnin V, van Belkum A, Hagen F, Meis JF, Durand G (2017) Routine identification of *Nocardia* species by MALDI-TOF mass spectrometry. Diagn Microbiol Infect Dis 87:7–10. https://doi.org/10.1016/j.diagmicrobio.2016.09.024
18. Body BA, Beard MA, Slechta ES, Hanson KE, Barker AP, Babady NE, McMillen T, Tang YW, Brown-Elliott BA, Iakhiaeva E, Vasireddy R, Vasireddy S, Smith T, Wallace RJ Jr, Turner S, Curtis L, Butler-Wu S, Rychert J (2018) Evaluation of the Vitek MS v3.0 matrix-assisted laser desorption ionization–time of flight mass spectrometry system for identification of *Mycobacterium* and *Nocardia* species. J Clin Microbiol. https://doi.org/10.1128/JCM.00237-18

19. Lebeaux D, Bergeron E, Berthet J, Djadi-Prat J, Mouniée D, Boiron P, Lortholary O, Rodriguez-Nava V (2019) Antibiotic susceptibility testing and species identification of *Nocardia* isolates: a retrospective analysis of data from a French expert laboratory, 2010-2015. Clin Microbiol Infect 25:489–495. https://doi.org/10.1016/j.cmi.2018.06.013
20. Rodriguez-Nava V, Couble A, Devulder G, Flandrois J-P, Boiron P, Laurent F (2006) Use of PCR-restriction enzyme pattern analysis and sequencing database for hsp65 gene-based identification of *Nocardia* species. J Clin Microbiol 44:536–546
21. CLSI (2008) MM18-A: interpretive criteria for identification of bacteria and fungi by DNA target sequencing, 1st edn. CLSI, Wayne
22. Yarbrough ML, Lainhart W, Burnham C-AD (2017) Identification of *Nocardia*, *Streptomyces*, and *Tsukamurella* using MALDI-TOF MS with the Bruker Biotyper. Diagn Microbiol Infect Dis 89:92–97. https://doi.org/10.1016/j.diagmicrobio.2017.06.019
23. Verroken A, Janssens M, Berhin C, Bogaerts P, Huang T-D, Wauters G, Glupczynski Y (2010) Evaluation of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of *Nocardia* species. J Clin Microbiol 48:4015–4021. https://doi.org/10.1128/JCM.01234-10
24. Khot PD, Bird BA, Durrant RJ, Fisher MA (2015) Identification of *Nocardia* species by matrix-assisted laser desorption ionization–time of flight mass spectrometry. Land GA, éditeur. J Clin Microbiol 53:3366–3369. https://doi.org/10.1128/JCM.00780-15
25. McTaggart LR, Chen Y, Poopalarajah R, Kus JV (2018) Incubation time and culture media impact success of identification of *Nocardia* spp. by MALDI-ToF mass spectrometry. Diagn Microbiol Infect Dis 92:270–274. https://doi.org/10.1016/j.diagmicrobio.2018.06.016
26. Tamura T, Ohji S, Ichikawa N, Hosoyama A, Yamazoe A, Hamada M et al (2018) Reclassification of *Nocardia* species based on whole genome sequence and associated phenotypic data. J Antibiot (Tokyo) 71:633–641
27. Gilquin JM, Riviere B, Jurado V, Audouy B, Kouatche JB, Bergeron E, Mouniée D, Molina T, Faure P, Saiz-Jimenez C, Rodríguez-Nava V (2016) First case of actinomycetoma in France due to a novel *Nocardia* species, *Nocardia boironii* sp. nov. mSphere 1(6). https://doi.org/10.1128/mSphere.00309-16
28. Iona E, Giannoni F, Brunori L, de Gennaro M, Mattei R, Fattorini L (2007) Isolation of *Nocardia asiatica* from cutaneous ulcers of a human immunodeficiency virus-infected patient in Italy. J Clin Microbiol 45:2088–2089. https://doi.org/10.1128/JCM.00263-07
29. Brown-Elliott BA, Killingley J, Vasireddy S, Bridge L, Wallace RJ Jr (2016) In vitro comparison of ertapenem, meropenem, and imipenem against isolates of rapidly growing *Mycobacteria* and *Nocardia* by use of broth microdilution and etest. J Clin Microbiol 54:1586–1592. https://doi.org/10.1128/JCM.00298-16