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ORIGINAL ARTICLE

Use of Andromas and Bruker MALDI-TOF MS in the identification of *Neisseria*

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Abstract
Through the past decade, MALDI-TOF MS has been recognized as a fast and robust tool for identification of most bacteria in clinical microbiology. However, the accuracy of this method to identify *Neisseria* species is still debated, and few data are available about commensal *Neisseria* species identification. In this study, we assessed two MALDI-TOF MS systems (Bruker Biotyper and Andromas) for the identification of 88, 18, and 29 isolates of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and commensal *Neisseria* species, respectively. All 88 isolates of *N. gonorrhoeae* were correctly identified using both systems, and most *N. meningitidis* and commensal *Neisseria* species were well identified: only 1/18 isolates of *N. meningitidis* was misidentified using Bruker Biotyper, and 1 isolate of *Neisseria polysaccharea* was misidentified as *N. meningitidis* using both systems. These results strengthen the possibility to use MALDI-TOF MS as a single method for *Neisseria* identification in routine, with excellent performance for *N. gonorrhoeae* identification. However, results should be interpreted prudently for *N. meningitdis* and commensal *Neisseria* species when isolated from genital and oropharyngeal samples where these both species can coexist.

Keywords Bacterial identification · *Neisseria meningitidis* · *Neisseria gonorrhoeae* · Commensal *Neisseria* species · Matrix-assisted laser desorption ionization-time of flight mass spectrometry · Bruker Biotyper MALDI-TOF · Andromas MALDI-TOF

Introduction

A fast and reliable identification of bacteria is crucial in clinical microbiology, particularly to distinguish closely related species displaying pathogenic or commensal phenotypes. Bacteria of the genus *Neisseria* illustrate this issue: this genus includes two major human pathogens, *Neisseria meningitidis* and *Neisseria gonorrhoeae*, and several commensal *Neisseria* species. All these species can colonize common anatomical sites, as upper respiratory and genital tract of humans [1], and reliable methods are needed to discriminate commensal and pathogenic *Neisseria* species.

Conventionally, species of genus *Neisseria* are distinguished on the basis of phenotypic or immunological properties, with methods displaying good performances for the identification of *N. meningitidis* and *N. gonorrhoeae* [2]. However, these methods have limits [2] and misidentifications between *N. gonorrhoeae* or *N. meningitidis* and other *Neisseria* species may potentially lead to serious health, legal, and social consequences [3, 4].

Conversely to conventional methods, matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) has been accepted during the last decade as a cost-effective method that provides reliable identification of most microorganisms isolated in a clinical microbiology laboratory [5, 6]. Recently, it has been suggested that MALDI-TOF MS displays good performances for

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N. gonorrhoeae identification [6–8] or *N. meningitidis* [6], but few studies have been interested in identification of commensal *Neisseria* species [5, 6, 9].

In our study, we compared Andromas and Bruker MALDI-TOF MS systems for the identification of a collection of *Neisseria* species including pathogenic and commensal *Neisseria*.

Materials and methods

A collection of 135 clinical isolates of *Neisseria* was used for this study. A total of 80 isolates of *N. gonorrhoeae* collected from the French National network (Renago) belonging to 32 different STs and 8 WHO isolates were included in the study. *N. meningitidis* and commensal *Neisseria* were recovered from bacterial collections at Henri Mondor (AP-HP, Créteil, France) and Lariboisière hospitals (AP-HP, Paris, France). Eighteen isolates of *N. meningitidis* belonging to B ($n = 5$), C ($n = 5$), A ($n = 2$), Y ($n = 2$), and W135 ($n = 3$) serogroups and one nonencapsulated isolate confirmed by the French national reference center at Pasteur Institute were included. A total of 29 commensal *Neisseria* species isolates, previously identified by 16S rDNA gene sequencing and additional genes detection (*opcA*, *ams*, *siaD*, *sodc*, *ctrA*) used as gold standards were studied [10]. This collection was composed by *N. subflava* group ($n = 13$), *N. mucosa* group ($n = 6$), *N. elongata* ($n = 4$), *N. cinerea* ($n = 2$), *N. bacilliformis* ($n = 1$), *N. polysaccharea* ($n = 1$), *N. weaveri* ($n = 1$), and *N. wadsworthii* (n = 1).

The isolates were cultured onto chocolate agar plates (bioMérieux, Marcy l'Etoile, France) and incubated at 37 °C in 5% CO_2 atmosphere for 24 and 48 h. Identifications were performed using LT2-Andromas (Beckman-Coulter, Villepinte, France) and Microflex Bruker Daltonics/BioTyper™ version 2.0 in parallel on the same cultures after 24 h and 48 h of incubation, according to the manufacturer's instructions. Each isolate was identified in duplicate, and the best identification score was retained. For the Bruker system, cutoff scores proposed by the manufacturers as previously described are 1.70–1.99 for the genus-level and > 2.00 for species-level identification [5, 6]. We established a threshold of 1.70 for species-level identification when the first three results were identical, as previously described [11]. For Andromas system, results were provided as a percentage of similarity between the sample spectrum and a database reference spectrum. Three categories of results were given: "good identification" (≥ 65% and difference between the first two species of at least 10%), "identification to be confirmed" (60–64%) and "no identification" (< 59% and/or difference < 10% between the first two species).

The species belonging to *N. subflava* group and *N. mucosa* group have been previously defined [12], and assignment under this level of identification has not been explored due to high rates of horizontal genetic exchanges within these groups [13].

Identification scores were compared using the *t* test and *p* values of < 0.05 were considered statistically significant. Analyses were performed using R, version 3.1.3.

Results

Identification of *N. gonorrhoeae* isolates Bruker and Andromas systems provided species-level identification for 100% (88/88) of isolates after 24 h and 48 h of incubation, respectively (Table 1, Fig. 1). Interestingly, no misidentification was detected for this diverse collection of *N. gonorrhoeae* on both systems, as observed in previous published data using Bruker [7, 8, 14] and Andromas systems [15].

Identification of *N. meningitidis* isolates For Bruker system, 94.4% (17/18) and 100% (18/18) of *N. meningitidis* isolates were correctly identified after 24 h and 48 h of incubation, respectively (Table 1). One isolate was misidentified as *N. elongata* with a low score of 1.84 by Bruker system at 24 h, but correctly identified as *N. meningitidis* (score 1.77) after 48 h of incubation. Interestingly, this misidentified isolate corresponds to a nonencapsulated *N. meningitidis* isolate.

For Andromas system, 88.9% (16/18) and 94.4% (17/18) of *N. meningitidis* isolates were correctly identified after 24 h and 48 h of incubation, respectively (Table 1). For the remaining isolates, 2/18 and 1/18 after 24 h and 48 h of incubation, respectively, no identification could be provided (Table 1, Fig. 1). Again, this lack of identification was provided for the nonencapsulated *N. meningitidis.*

Identification of commensal *Neisseria* species Using Bruker system, 93.1% (27/29) and 86.2% (25/29) of commensal *Neisseria* isolates were correctly identified after 24 h and 48 h of incubation, respectively (Table 1). Using Andromas system, a correct identification was obtained for 89.7% (26/29) and 93.1% (27/29) of isolates after 24 h and 48 h of incubation, respectively (Table 1). No identification was obtained for one *N. wadsworthii* isolate for the two time-points, using both systems. Noteworthy, no spectrum of this species is present in databases of both systems. At least, one *N. polysaccharea* isolate was misidentified as *N. meningitidis* at 24 h and 48 h by Bruker (with a score of 2.1 and 1.76, respectively). The same isolate was not identified at 24 h, and was misidentified as *N. meningitidis* at 48 h by Andromas system (75% likelihood) (Table 1, Fig. 1).

Impact of time of incubation on score values (Fig. 1) For *N. gonorrhoeae* isolates, the score of identification was significantly lower for the Andromas system after 48-h

Table 1 Identification of *Neisseria* species isolates using Bruker and Andromas MALDI-TOF systems after 24 h and 48 h of culture

Neisseria species	No. of isolates	Bruker system						Andromas system					
		24 h			48 h			24 h			48 h		
		SLI[a]	MisID[a]	No. ID[a]	SLI	MisID	No. ID	SLI	MisID	No. ID	SLI	MisID	No. ID
N. gonorrhoeae	88	88 (100)			88 (100)		2	88 (100)			88 (100)		
N. meningitidis	18	17 (94.4)	1[b]		18 (100)			16 (88.9)		2	17 (94.4)		1
Commensal Neisseria	29	27 (93.1)	1	1	25 (86.2)	2	2	26 (89.7)	1	2	27 (93.1)	1	1
N. subflava group	13	13			13			12	1[c]		13		
N. mucosa group	6	6			6			6			6		
N. elongata	4	4			3	1[d]		4			4		
N. cinerea	2	2			1		1	2			2		
N. bacilliformis	1	1			1			1			1		
N. polysaccharea	1		1[e]			1[e]				1		1[e]	
N. weaveri	1	1			1			1			1		
N. wadsworthii	1			1			1			1			1

[a] SLI: species-level identification, MisID: misidentification, NoID: no identification

[b] One *N. meningitidis* isolate misidentified as *N. elongata* (score 1.84)

[c] One *N. subflava* group isolate misidentified as *N. mucosa* group (72% likelihood)

[d] One *N. elongata* isolate was misidentified as *N. subflava* group (score 2.13)

[e] One *N. polysaccharea* isolate was misidentified as *N. meningitidis* at 24 h and 48 h by Bruker (with a score of 2.1 and 1.76, respectively). The same isolate was not identified at 24 h, and was misidentified as *N. meningitidis* at 48 h by Andromas (75% likelihood)

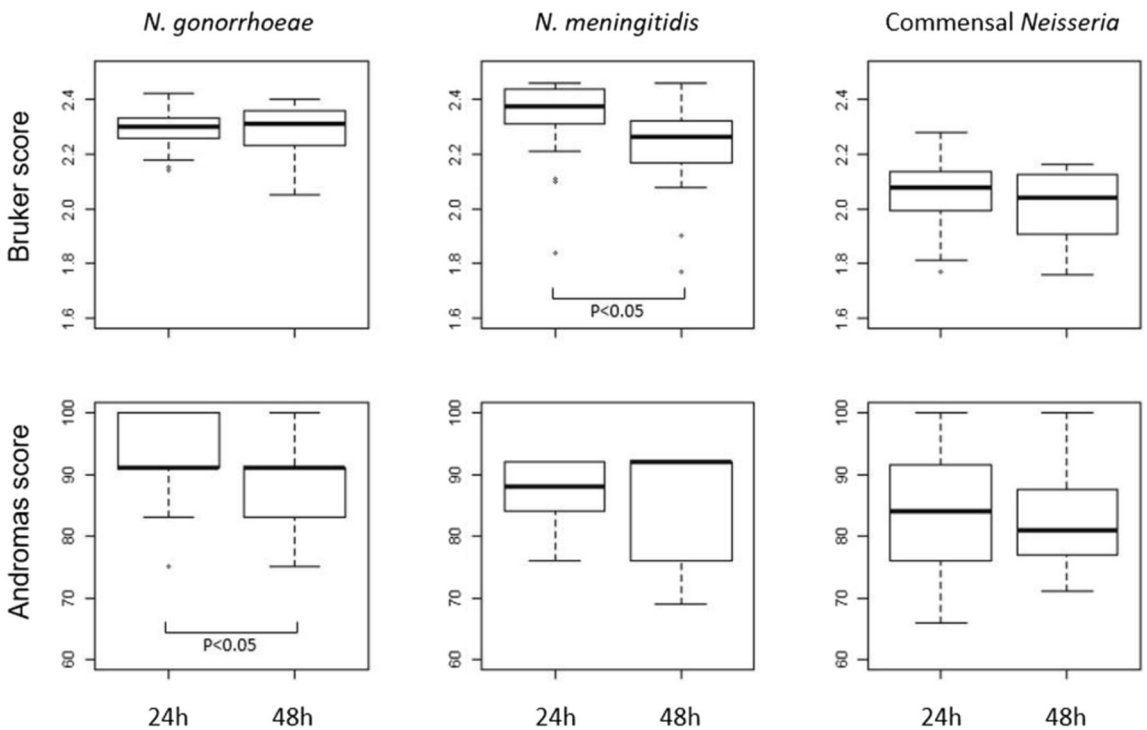

Fig. 1 Box plot scores using Bruker and Andromas MALDI-TOF MS systems for Neisseria isolates identification after 24 h and 48 h of incubation. Cutoff identification scores: Bruker system: species-level > 2.00 or 1.70 with the first three results identical; genus-level 1.70–1.99. Andromas system: "good identification" ≥ 65%; "identification to be confirmed" 60–64%

incubation ($p < 0.05$). If no misidentification was evidenced, this result suggests that this system is more sensitive to time of incubation. Conversely, the Bruker system displayed lower scores for *N. meningitidis* after 48 h of incubation ($p < 0.05$).

Discussion

During the last decade, MALDI-TOF MS has been accepted as a reliable tool for identification of most bacteria cultured in routine in clinical microbiology. However, few data are available for identification of *Neisseria* by MALDI-TOF MS systems, and reliability of such systems is still matter of debate.

For both systems, a correct identification was found for 100% of 88 *N. gonorrhoeae* isolates, including 8 WHO isolates and 80 isolates with diverse genetic background collected from the French National network. These results are in line with previous published data using Bruker [7, 8, 14] and Andromas systems [15].

Both systems were able to discriminate most of 18 *N. meningitidis* and 29 commensal *Neisseria* species isolates, but two major misidentifications have been evidenced. In our study, one isolate of a nonencapsulated *N. meningitidis* was misidentified as *N. elongata* (score 1.84) by Bruker system at 24 h, but correctly identified as *N. meningitidis* (score 1.77) after 48 h of incubation (Table 1). No identification was obtained for this isolate using Andromas system. Previous studies have shown that *N. meningitidis*, especially nonencapsulated isolates, and commensal *Neisseria* species, especially *N. polysaccharea*, can be hard to distinguish [16]. However, misidentification of this nonencapsulated *N. meningitidis* isolate as *N. elongata*, is quite surprising considering the relative phylogenetic distances between these two species [12] and its isolation from a case of meningitidis in an immunosuppressed patient.

Conversely, one isolate of *N. polysaccharea* of our collection was misidentified as *N. meningitidis* at 24 h (score 2.1) and 48 h (score 1.76) by Bruker and at 48 h by Andromas (score = 75%) (Table 1). As seen before, misidentification of *N. polysaccharea* in *N. meningitidis* has been previously described [10, 17, 18], probably due to close phylogenetic relatedness of *N. polysaccharea* and *N. meningitidis* [12]. More basically, misidentification of commensal *Neisseria* species as *N. meningitidis* have been reported with Andromas [15] and Shimadzu/SARAMIS MALDI-TOF MS systems [6].

Concerning the identification of commensal *Neisseria* species, about one third of isolates displayed a score of identification between 1.7 and 2.0 for Bruker system (Fig. 1) but the threshold of 1.70 was adequate for species-level identification as previously described [11]. Interestingly, reliability of identification was not systematically associated with this threshold modification.

Finally, our study has evidenced that time of incubation impacted significantly score values for the identification of *N. gonorrhoeae* isolates with the Andromas system and for *N. meningitidis* with Bruker system. These two dissimilarities are probably due to differences in engineering of both systems, based on different MALDI-TOF apparatus, different databases, and different algorithms.

As a conclusion, we showed that *N. gonorrhoeae* were well identified by both systems, reinforcing the possibility to use MALDI-TOF MS as a single method for *N. gonorrhoeae* identification in routine [7]. For commensal *Neisseria* species and *N. meningitidis*, correct identifications were obtained for most tested isolates. However, identification must be considered prudently, particularly for strains isolated in oropharyngeal or genital microbiota, potentially colonized by commensal *Neisseria* species and *N. meningitidis*. These results underline the need for better databases or processes of protein extractions [9], or complementary tests for accurate identification of these species.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Not applicable.

Informed consent Not applicable.

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