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Identification of Clinical *Streptococcus pneumoniae* Isolates among other Alpha and Nonhemolytic Streptococci by Use of the Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System

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Discrimination between *Streptococcus pneumoniae* and its close relatives of the viridans group is a common difficulty in matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry-based identification. In the present study, the performances of the Vitek MS MALDI-TOF mass spectrometry system were assessed using 334 pneumococci, 166 other *S. mitis* group streptococci, 184 non-*S. mitis* group streptococci, and 19 related alpha- and nonhemolytic aerobic Gram-positive catalase-negative coccal isolates. Pneumococci had been identified by means of optochin susceptibility and bile solubility or serotyping, and other isolates mainly by use of RapidID32 Strep strips. In case of discordant or low-discrimination results, genotypic methods were used. The sensitivity of the Vitek MS for the identification of *S. pneumoniae* was 99.1%, since only three bile-insoluble isolates were misidentified as *Streptococcus mitis*/*Streptococcus oralis*. Conversely, two optochin-resistant pneumococci were correctly identified (specificity, 100%). Three *Streptococcus pseudopneumoniae* isolates were also correctly identified. Among nonpneumococcal isolates, 90.8% ($n = 335$) were correctly identified to the species or subspecies level and 2.4% ($n = 9$) at the group level. For the remaining 25 isolates, the Vitek MS proposed a bacterial species included in the list of possible species suggested by genotypic methods, except for 4 isolates which were not identified due to the absence of the species in the database. According to our study, the Vitek MS displays performance similar to that of the optochin susceptibility test for routine identification of pneumococcal isolates. Moreover, the Vitek MS is efficient for the identification of other viridans group streptococci and related isolates, provided that the species are included in the database.

Streptococcus pneumoniae is responsible for upper respiratory tract infections (otitis and sinusitis) as well as severe diseases (pneumonia, bacteremia, and meningitis) associated with high morbidity and mortality rates (1). More than 90 capsular serotypes have been characterized, with an unequal distribution in systemic infections (2). The species *S. pneumoniae* belongs to the *S. mitis* group streptococci, which are part of the so-called viridans streptococci group, which also includes the *S. salivarius*, *S. mutans*, *S. anginosus*, and *S. bovis* groups (1, 3). In contrast to pneumococci, nonpneumococcal viridans group streptococci colonizing the upper respiratory tract are rarely responsible for systemic infections like endocarditis (1). Given these differences in clinical significance, accurate identification of *S. pneumoniae* is essential.

Both the biochemical systems and the commercial antigen detection tests available for pneumococcal identification (ID) are recognized as unsatisfactory (1, 4). The simplest and most reliable tests for *S. pneumoniae* ID in clinical laboratories are optochin susceptibility and/or bile solubility. However, optochin-resistant and bile-insoluble pneumococcal isolates and optochin-susceptible nonpneumococcal alpha-hemolytic streptococci have been reported (1, 4–9). Another pitfall of using optochin susceptibility testing is the differentiation of *S. pseudopneumoniae* isolates (10). Detection and determination of capsular polysaccharides of *S. pneumoniae* with type-specific antisera is a valuable second-line ID test but remains mainly an epidemiologic tool and entails the problem of untypeable pneumococci (1, 11, 12). To overcome problems in phenotypic pneumococcal ID, pneumococcus-specific PCRs targeting anonymous DNA fragments or genes encoding virulence factors like pneumolysin (*plyA*), autolysin (*lytA*), an oxidative stress resistance component (*psaA*), and capsular biosynthesis (*cpsA*) have been developed (13–20). Partial sequence analysis or specific nucleotide signatures of various housekeeping genes have been also proposed as an alternative approach for discriminating pneumococci and viridans streptococci (21–26). However, *S. pneumoniae* and other *S. mitis* group streptococci may share a high degree of DNA sequence identity due to the high frequency of genetic transformation of these bacterial species (27–29). In addition, these numerous molecular methods, which display variable specificity and reliability, remain inconvenient for routine diagnostic use.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS)-based systems are increasingly replacing conventional phenotypic methods for routine ID of bacteria due to their fast, easy-to-use, cost-effective, and thus high-throughput performances (30–37). However, one of the main limitations reported for MALDI-TOF MS systems remains the

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lack of discrimination between S. *pneumoniae* and other closely related alpha-hemolytic streptococci (30, 33, 36–40). Conversely, two previous studies from Martiny and coworkers and our laboratory tested the Vitek MS (bioMérieux, France), a MALDI-TOF mass spectrometry-based ID system, and reported the apparent appropriate species ID of *S. pneumoniae* and other viridans group streptococci. Nonetheless, these studies were based on only a few isolates (34, 35). In the present study, we evaluated the performances of the Vitek MS system on a large and representative clinical collection of 334 pneumococci, 166 other *S. mitis* group streptococci, 184 non-*S. mitis* group streptococci, and 19 related alpha- and nonhemolytic aerobic Gram-positive catalase-negative coccal isolates.

MATERIALS AND METHODS

Bacterial isolates. A set of 334 nonredundant isolates of *S. pneumoniae* recovered in the Midi-Pyrénées region of France in 2009 as part of the regional epidemiological surveillance of *S. pneumoniae* ($n = 300$) or randomly chosen from our 2011 laboratory collection ($n = 34$) were analyzed (2). Pneumococcal strains originated from 193 blood cultures, 55 acute otitis media samples, 38 respiratory samples, 28 cerebrospinal fluids, 15 pleural effusions, two conjunctivitis samples, one joint fluid sample, one vaginal sample, and one skin lesion. Moreover, all nonredundant nonpneumococcal isolates recovered in our laboratory during the first 9 months of 2011 ($n = 369$, except 30 *Streptococcus anginosus* and 30 *Streptococcus constellatus* isolates randomly chosen), encompassing 166 other *S. mitis* group and 184 other non-*S. mitis* group viridans group streptococci, as well as 19 isolates belonging to six related genera were also included in this study. All isolates were cultured on 5% sheep blood Columbia agar (bioMérieux) for 18 h to 48 h at 35°C under a 5% CO_2 atmosphere.

Optochin susceptibility test. A 5-μg optochin disk (6-mm disks; MAST ID Optochin Discs, MAST, United Kingdom) was placed on sheep blood Columbia agar plates inoculated under 5% CO_2 atmosphere, or ambient atmosphere when stated. Optochin susceptibility was defined as an inhibition zone of ≥14 mm.

Bile solubility test. The bile solubility test was performed with the tube method, with preparation of bacterial suspensions in 1 ml of 0.9% NaCl equivalent to a McFarland 1.0 standard. A 0.5-ml portion of 2% deoxycholate was added to a 0.5-ml suspension of each isolate prepared in 0.9% NaCl and incubated at 35°C for 1 h. A positive test was indicated by visible clearing of the suspension. A negative control for each isolate was similarly performed with 0.5 ml of 0.9% NaCl added to a 0.5 ml suspension of each isolate prepared in 0.9% NaCl.

Serotyping method. Pneumococcal capsular polysaccharide serotype was determined by agglutination with specific antisera (Statens Serum Institut, Copenhagen, Denmark).

Phenotypic identification systems. Rapid ID32 Strep strips or the Vitek 2 system using the GP card (bioMérieux) were performed according to the manufacturer's instructions, including complementary tests if required. For the Rapid ID32 Strep strips, reading of the colorimetric reactions was achieved by the mini-API instrument (bioMérieux).

DNA extraction and molecular methods. Template DNA was prepared by suspending a loopful of colonies in 200 μl of 0.1 M Tris-EDTA. Two microliters of 5 kU/ml of mutanolysin (Sigma) was added to 20 μl of the suspension, and the mixture was incubated at 56°C for 30 min, boiled for 10 min, and then centrifuged. Two microliters of the supernatant was used as the template in PCR mixtures. *ply* (18), *lytA* (14), *psaA* (15), and MFP (17) PCR assays (all specific to pneumococci), amplifying, respectively, a 209-bp, 101-bp, 838-bp, and 181-bp fragment, were performed as described previously. Partial sequencing of the *recA* gene (313 bp), encoding a recombinase subunit, of the *sodA* gene (435 bp), encoding the manganese-dependent superoxide dismutase, of the *gyrB* gene (458 bp), encoding the B subunit of DNA gyrase, and of the 3′-terminal part of the 16S rRNA gene (440 bp) was carried out using the primer pairs *recA*2F/*recA*5R, d1/d2, streptogyrBd/streptogyrBr, and MFP889/MFP890, respectively, as previously described (17, 26, 41). Searches for sequence homologies were carried out using the BIBI service combined with the GenBank database and the Basic Local Alignment Search Tool. 16S rRNA gene and *recA* gene sequence identities of ≥97.0% to the first proposed sequence of a classified species with a demarcation of 2% to the second classified species was considered to assign an ID to the species level (26, 42). Similarly, sequence identities of ≥94.0% for *sodA* and *gyrB* with a demarcation of 2% were arbitrarily used, as no criteria had yet been proposed.

MALDI-TOF MS identification. Plate preparation, mass spectrum generation, and processing were performed with the Vitek MS system (bioMérieux) as previously described, using an Axima Assurance mass spectrometer with version 1.0.0 of the acquisition software and MS-ID database (34). According to the manufacturer's instructions, a confidence value from 60 to 99.9% with a single species proposed was considered a good ID. If a unique ID pattern was not recognized, a list of possible organisms was given corresponding to a low-discrimination result (LD) when a confidence value was >60% for each proposed species. When confidence values were <60%, the strain was determined to be outside the scope of the database and the result was recorded as "no ID." In the case of warning messages generated by poor-quality spectra, LD results, and no-ID results, the deposits were read again, and when required, the isolates were tested again with a single deposit. In case of persistent LD results or poor-quality spectra, a protein extraction step was performed, as previously described (43).

Reference identifications and result management. Presumptive pneumococcal isolates were tested for optochin susceptibility and bile solubility or serotype. *S. pneumoniae* isolates were defined as optochin susceptible (inhibition zone of ≥14 mm) under a CO_2 atmosphere and bile soluble or typeable. Bile-insoluble or nontypeable isolates exhibiting an optochin inhibition zone of >6 mm and <14 mm under a CO_2 atmosphere but of ≥14 mm under an ambient atmosphere in a complementary test were considered *S. pseudopneumoniae* (10). Other isolates were identified using the Rapid ID32 Strep system, except for *S. bovis* group and *Helcococcus kunzii* isolates, which were identified using the Vitek 2 system. *S. mitis* group isolates were confirmed to be optochin resistant.

In the case of discordant results between Vitek MS and conventional phenotypic methods, or low-discrimination results with conventional phenotypic methods, including discordant results between optochin susceptibility tests and bile solubility tests or serotypeability, or absence of ID (no ID) with the Vitek MS system, genetic IDs were performed, and the results were considered the reference ID. For management of discordant results, different partial gene sequencing was performed according to presumptive ID: *recA* sequencing, currently one of the most reliable genotypic tools for proper ID to species level of pneumococci and closest related species (26); 16S rRNA gene sequencing for catalase-negative nonstreptococcal isolates; and *sodA* sequencing for others. For informative purposes, partial *gyrB* gene sequencing was also performed in some cases with inconclusive IDs according to *sodA* sequencing. Moreover, bile solubility testing was carried out, along with *ply*-, *lytA*-, *psaA*-, and MFP-specific PCR assays for discrimination between *S. pneumoniae* and the most closely related species, for informative purposes.

RESULTS AND DISCUSSION

Technical efficiency. MALDI-TOF MS-based IDs were efficiently carried out with a unique deposit for 94.3% of the isolates ($n = 663$; Table 1), including 17 of 22 (77%) mucoid pneumococcal isolates. This technical efficiency for analysis of Gram-positive cocci with a single deposit without an extraction procedure is consistent with previous reports regarding the Vitek MS and Andromas MALDI-TOF MS-based systems in contrast to the Biotyper system (Bruker) (30, 31, 34, 35, 37, 39). A second deposit performed with 5.7% of the isolates ($n = 40$) resolved half of the IDs (Table 1). The six persistent no-ID results were considered

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TABLE 1 Distribution of result categories among all 703 isolates tested, according to identification attempts

Result	No. of isolates (%) with result after:		
	Single deposit	Second deposit	Extraction step
Good ID	663 (94.3)[a]	20 (2.8)	13 (1.9)[b]
LD	17 (2.4)	9 (1.3)	1 (0.1)[c]
No ID	13 (1.9)	6 (0.9)	ND[d]
Warning messages	10 (1.4)	5[b] (0.7)	0
Total	703 (100)	40 (5.7)	14 (2.0)

[a] Including 17 of 22 (77%) mucoid pneumococcal isolates.
[b] Corresponding to or including 5 of 22 (23%) mucoid pneumococcal isolates.
[c] Corresponding to an LD result between *Gemella morbillorum* and *Gemella hemolysans*.
[d] ND, not done (after two deposits with a no-ID result, the isolates were considered outside the scope of the database).

true absences of ID. An extraction step followed by a unique deposit was performed for persistent LD results ($n = 9$) and poor-quality spectra ($n = 5$); the latter corresponded to mucoid pneumococci that account for 23% of all the mucoid pneumococcal isolates tested. The extraction step resolved all IDs but one, which remained an LD between *Gemella morbillorum* and *Gemella haemolysans*. Low-confidence values, LD results, and requirement of a second deposit or of an extraction step were not related to particular species, except that 23% of mucoid pneumococcal isolates needed an extraction step.

Inconsistent results occurred for three isolates, identified as coagulase-negative staphylococci or β-hemolytic *Streptococcus*. Performing a second deposit allowed us to successfully identify two isolates, the other one belonging to a species that was outside the scope of the database. These outright inconsistent IDs emphasize the fact that bacteriological technical expertise remains essential to avoid major misidentifications.

***S. pneumoniae* identification performances.** A large diversity of pneumococci was assessed in this study, which includes 267 isolates belonging to 22 different serogroups. The sensitivity of the Vitek MS system for the ID of *S. pneumoniae* was 99.1%, with three of the 334 isolates being misidentified as *Streptococcus mitis/oralis* (Table 2) according to partial *recA* gene sequencing analysis. These three misidentified *S. pneumoniae* were isolated from respiratory samples and are atypical pneumococci, since all three isolates were bile insoluble, and two of them were negative for MFP and *psaA* amplifications. These PCRs have been recently reported to be among the most reliable species-specific PCRs to appropriately discriminate *S. pneumoniae* and the most closely related species, like *S. pseudopneumoniae* (13, 17). Conversely, the specificity of the Vitek MS system for *S. pneumoniae* ID was 100% (Table 2), with two optochin-resistant, bile-soluble, and typeable pneumococci correctly identified. One of these two isolates, originating from a blood culture, was *psaA* and *lytA* negative. In contrast to previous reports related to other MALDI-TOF systems (30, 33, 36–40), the Vitek MS system allows good discrimination between the *S. pneumoniae* species and other viridans streptococcal species. This is probably linked to its particular algorithm, which may efficiently detect the specific mass/charge peak profiles of *S. pneumoniae* and the most closely related species, as recently highlighted by Werno and coworkers (44).

Of note, the good performances of the Vitek MS for the ID of *S. pneumoniae* were obtained from pure culture on agar media. Viridans group streptococci form minuscule to small colonies, frequently requiring the use of more than one colony to perform the deposit of intact whole bacteria on the MALDI-TOF MS target. Since viridans group streptococci can have similar colony morphologies, performing a deposit with more than one colony to detect *S. pneumoniae* isolates directly from a possibly polymicrobial culture, like those frequently originated from respiratory samples, remains very hazardous.

Nonpneumococcal identification performances. Among nonpneumococcal isolates, 90.8% ($n = 335$) were correctly identified to the species or subspecies level (Table 2). Isolated from respiratory samples without any other pathogenic bacteria recovered, all three *S. pseudopneumoniae* isolates were correctly identified. The three isolates were *psaA* negative, and two isolates were *ply* positive, which is consistent with the reported specificity of the *psaA* PCR for *S. pneumoniae* ID and the frequent *ply*-positive PCRs among *S. pseudopneumoniae* isolates (10, 13, 45).

Four nonpneumococcal isolates (1.1%) were not identified due to the absence of these species in the database (*Streptococcus massiliensis*, *Facklamia tabacinasalis*, *Facklamia ignava*, and *Aerococcus sanguinicola*) (Table 2). The *sodA* gene sequence of the *S. massiliensis* isolate displayed only 94.2% identity with the sequence of the reference strain 4401825, which harbors a 27-bp duplication. In contrast, the partial *gyrB* sequence showed 100% identity with the one of the reference strain (46). The partial *sodA* and *gyrB* sequences of this *S. massiliensis* isolate from peritoneal fluid had been deposited in the EMBL Nucleotide Sequence Database into the accession numbers HF677578 and HF677579, respectively.

Nine nonpneumococcal isolates (2.4%) were correctly identified to the group level but misidentified at the species or subspecies level, including five *S. mitis* group, one *S. anginosus* group, and three *S. bovis* group isolates. The five *S. mitis* group isolates were five *Streptococcus australis* isolates identified as *Streptococcus parasanguinis* or *Streptococcus sanguinis* (Table 2). These *S. australis* misidentifications may be due to the close relationships of *S. australis* with *S. sanguinis* and *S. parasanguinis* (46), together with the absence of *S. australis* in the database of the Vitek MS.

Reference IDs using genotypic methods were obtained only at the group level for 5.7% ($n = 21$) of nonpneumococcal isolates, including five *S. mitis* group, nine *S. anginosus* group, five *S. bovis* group, and two related-genus isolates. The Vitek MS proposed a bacterial species included in the list of possible species suggested by *sodA* or 16S rRNA gene sequence analysis for 19 isolates and no ID for two isolates (Table 2). The latter were an *S. mitis* group isolate for which the *sodA* gene sequence was similar to *S. mitis* group species absent from the Vitek MS database (*S. australis*, *S. infantis*, and *S. peroris*), and a *Granulicatella* isolate for which partial 16S rRNA gene sequencing suggested species present in the Vitek MS database (*G. adiacens* and *G. elegans*) or absent in the Vitek MS database ("*Granulicatella para-adiacens*").

In the *S. anginosus* group, considerable genetic similarity has been previously reported, which adds to the complexity of defining species, despite an apparent biochemical heterogeneity (1, 47, 48). By sequencing four different housekeeping genes, Glazunova and coworkers showed that some *S. anginosus* group isolates were identified as either *S. anginosus* or *S. constellatus*, depending on which gene sequence was analyzed (47). For informative purpose, *gyrB* gene sequencing was performed for two randomly chosen isolates. The *gyrB* gene sequence confirmed the identification ob-

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TABLE 2 Relevant phenotypic and genotypic test results for the 703 strains studied

Reference ID (no. of isolates)[a]	Vitek MS ID	Optochin CO_2[b]	BS test[c]	Pneumococcal capsule identification	PCR result				No. of isolates with sequencing			No. of isolates (%) with Vitek MS			
					psaA	MFP	*ply*	*lytA*	*recA*	*sodA*	16S rRNA	Correct ID	Incorrect ID	Unresolved ID	No ID
Pneumococcal isolates (334)												331 (99.1)	3 (0.9)		
S. pneumoniae (292)	*S. pneumoniae*	S	ND	Yes								292			
S. pneumoniae (37)	*S. pneumoniae*	S	Soluble	No or ND[d]								37			
S. pneumoniae (1)	*S. pneumoniae*	R (R)	Soluble	3	+	+	+	+	1			1			
S. pneumoniae (1)	*S. pneumoniae*	R (R)	Soluble	23F	−	+	+	−	1			1			
S. pneumoniae (1)	*S. mitis*/*S. oralis*	S (S)	Not soluble	23	+	+	+	+	1				1		
S. pneumoniae (1)	*S. mitis*/*S. oralis*	S (S)	Not soluble	37	−	−	+	+	1				1		
S. pneumoniae (1)	*S. mitis*/*S. oralis*	S (S)	Not soluble	ND	−	−	+	+	1				1		
Nonpneumococcal isolates (369)												335 (90.8)	9 (2.4)	21 (5.7)	4 (1.1)
Other mitis group streptococci (166)												155 (93.4)	5 (3)	5 (3)	1 (0.6)
S. pseudopneumoniae (1)	*S. pseudopneumoniae*	R (S)	Not soluble	No	−	ND	+	+	1			1			
S. pseudopneumoniae (1)	*S. pseudopneumoniae*	R (S)	Not soluble	ND	−	ND	+	+	1			1			
S. pseudopneumoniae (1)	*S. pseudopneumoniae*	R (S)	Not soluble	ND	−	ND	−	−	1			1			
S. mitis/*S. oralis* (126)[e]	*S. mitis*/*S. oralis*	R										126			
S. gordonii (7)	*S. gordonii*	R								4		7			
S. cristatus (1)	*S. cristatus*	R								1		1			
S. sanguinis (7)	*S. sanguinis*	R								3		7			
S. parasanguinis (11)	*S. parasanguinis*	R								1		11			
S. australis (3)[f]	*S. parasanguinis*	R								3			3		
S. australis (2)[f]	*S. sanguinis*	R								2			2		
S. massiliensis (1)[f]	No ID	R								1					1
S. oralis/*S. parasanguinis* (1)	*S. parasanguinis*	R								1				1	
S. mitis group (4)	2 *S. mitis*/*oralis*, 1 *S. parasanguinis*, 1 no ID	R	Not soluble							4				4	
Non-mitis group streptococci (184)												166 (90.2)	4 (2.2)	14 (7.6)	
S. salivarius group (39)												39 (100)			
S. salivarius subsp. *salivarius* (35)	*S. salivarius*									2		35			
S. vestibularis (4)	*S. vestibularis*									3		4			
S. mutans group (2)												2 (100)			
S. mutans (2)	*S. mutans*											2			
S. anginosus group (111)												101 (91)	1 (0.9)	9 (8.1)	
S. constellatus (37)	*S. constellatus*									2		37			
S. anginosus (48)	*S. anginosus*									5		48			
S. constellatus/*S. anginosus* (5)	*S. constellatus*									5				5	
S. constellatus/*S. anginosus* (4)	*S. anginosus*									4				4	
S. anginosus (1)	*S. constellatus*									1			1		
S. intermedius (16)	*S. intermedius*											16			
S. bovis group (32)												24 (75)	3 (9.4)	5 (15.6)	
S. gallolyticus subsp. *gallolyticus* (4)	*S. gallolyticus* subsp. *gallolyticus*											4			
S. gallolyticus subsp. *pasteurianus* (18)	*S. gallolyticus* subsp. *pasteurianus*											18			
S. gallolyticus subsp. *pasteurianus* (2)	*S. gallolyticus* subsp. *gallolyticus*									2			2		
S. bovis (1)	*S. bovis*											1			
S. lutetiensis/*S. bovis*/*S. equinus* (1)	*S. bovis*									1				1	
S. lutetiensis/*S. bovis*/*S. equinus* (4)	*S lutetiensis*									4				4	

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S. lutetiensis/*S. bovis*/*S. equinus* (1)	*S. gallolyticus* subsp. *gallolyticus*			1			1			
S. infantarius subsp. *infantarius* (1)	*S. infantarius* subsp. *infantarius*			1		1				
Related genera (19)						14 (73.7)		2 (10.5)	3 (15.8)	
Abiotrophia defectiva (1)	*A. defectiva*					1				
Granulicatella adiacens (1)	*G. adiacens*					1				
"*Granulicatella para-adiacens*"[f]/*G. adiacens*/*G. elegans* (1)	No ID			1				1		
Gemella morbillorum/*G. haemolysans* (1)	*G. morbillorum*/*G hemolysans*			1				1		
Helcococcus kunzii (2)	*Helcococcus kunzii*					2				
Facklamia tabacinasalis (1)[f]	No ID			1					1	
Facklamia ignava (1)[f]	No ID			1					1	
Aerococcus viridans (2)	*A. viridans*					2				
Aerococcus urinae (8)	*A. urinae*					8				
Aerococcus sanguinicola (1)[f]	No ID			1					1	
Total (703)		8	51	5	666 (94.7)	12 (1.7)	21 (3)	4 (0.6)		

[a] BS test, desoxycholate solubility test.
[b] Reference identifications were obtained using Rapid ID32 Strep strips or the VITEK2 system by default, or with gene sequencing when stated.
[c] S, susceptible; R, resistant. Results were obtained under an atmosphere of increased CO_2; parentheses indicate a result obtained under an ambient atmosphere.
[d] Including three polyagglutinable isolates and 12 nonagglutinable isolates. ND, not determined.
[e] Including 52 *S. oralis*, 68 *S. mitis*, and 6 *S. mitis*/*S. oralis* isolates.
[f] Species absent from the Vitek MS database.

tained with the MALDI-TOF MS (*S. constellatus*) for only one of them.

Hinse and coworkers recently reported the reliability of MALDI-TOF MS technology for the ID of almost all members of the *S. bovis* group streptococci in comparison to *sodA* gene sequencing analysis (49). The authors also highlighted the fact that in this group, which has undergone many taxonomic changes over the last decade (50, 51), some strains were mislabeled in collection databases, or that species or subspecies had been assigned in nucleotide databases prior to the latest taxonomic revision of this species complex. Accordingly, IDs proposed by the Vitek MS for the eight isolates that were misidentified or had unresolved IDs could be correct (Table 2), unless the Vitek MS database was constructed with some mislabeled strains.

In conclusion, according to our study, the Vitek MS displays performances similar to that of the optochin susceptibility test for routine ID of pneumococcal isolates. Provided that the species are included in the database, this system is an efficient method for the ID of nonpneumococcal viridans streptococci to the group level and in most cases to the species level.

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