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Performance of the VITEK MS v2.0 system in distinguishing *Streptococcus pneumoniae* from non-pneumococcal species of the *Streptococcus mitis* group

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Abstract

The VITEK MS v2.0 MALDI-TOF mass spectrometry system accurately distinguished *S. pneumoniae* from non-pneumococcal *S. mitis* group species. Only 1 of 116 non-pneumococcal isolates (<1%) was misidentified as *S. pneumoniae*. None of 95 pneumococcal isolates was misidentified. This method provides a rapid, simple means of discriminating among these challenging organisms.

Short-form paper

Using conventional phenotypic identification methods, it has been challenging for clinical laboratories to distinguish accurately between bacterial species within certain groups, such as the coagulase-negative staphylococci or the nonfermenting gram-negative bacilli. The *Streptococcus mitis* group is another set of closely-related species between which conventional identification methods cannot reliably differentiate. The most important pathogen within the *S. mitis* group, *S. pneumoniae*, is conventionally distinguished from the others (*S. mitis*, *S. oralis*, *S. pseudopneumoniae*, *S. sanguinis*, *S. parasanguinis*, *S. gordonii*, *S. cristatus*, *S. infantis*, *S. peroris*, *S. australis*, *S. sinensis*, *S. orisratti*, *S. oligofermentans*, and *S. massiliensis*) based on its susceptibility to optochin or its solubility in bile. However, both the sensitivity and specificity of optochin susceptibility testing are suboptimal. Some *S. pneumoniae* strains are optochin resistant [1-3], and closely related species such as *S. pseudopneumoniae* or *S. mitis* can exhibit optochin susceptibility, particularly when incubated in ambient air rather than CO_2-enriched air [4-8]. Likewise, the most convenient method of bile solubility testing, the plate method, is relatively non-specific [9] and some strains of *S. pneumoniae* are bile

insoluble even by the tube method [10] or the disk method [11]. Even when larger batteries of phenotypic tests are applied, such as the API rapid ID 32 Strep strip or the VITEK 2 GP card (bioMérieux, Marcy l'Etoile, France), discrimination among species within the *S. mitis* group is poor [12]. In fact, *S. mitis* group species are so closely related that the AccuProbe *Streptococcus pneumoniae* assay (Hologic Gen-Probe, Inc., San Diego, CA), a commercially-available DNA probe hybridization test, cannot differentiate between *S. pneumoniae* and *S. pseudopneumoniae* isolates [4, 6], and 16S rRNA gene sequencing cannot reliably distinguish between *S. pneumoniae*, *S. mitis* and *S. oralis* [13, 14].

Recent investigations have demonstrated the ability of matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) to distinguish between closely related bacterial species with a high degree of confidence [15-23]. Yet with regard to the *S. mitis* group species initial reports have been disappointing, inasmuch as one widely-used, commercially-available MALDI-TOF MS platform is prone to misidentify *S. mitis*, *S. oralis* or *S. pseudopneumoniae* as *S. pneumoniae* [7, 11, 24-29]. However, other commercial platforms may perform differently in this regard. In particular, a recent multi-center evaluation of the bioMérieux VITEK MS v2.0 system demonstrated accurate separation between 51 *S. pneumoniae* strains and 71 non-pneumococcal strains from the *S. mitis* group, although for one *S. mitis* isolate the system did report a split identification that included *S. pneumoniae* among the alternatives [30]. Here, we used a larger collection of *S. mitis* group clinical isolates to assess the performance of the bioMérieux VITEK MS v2.0 system in differentiating *S. pneumoniae* from other *S. mitis* group species.

The study included 211 *S. mitis* group clinical isolates selected from frozen archives at Massachusetts General Hospital. None of the study isolates overlapped with those entered into the recent multi-center evaluation of the bioMérieux VITEK MS v2.0 system [30]. In our laboratory, all clinical isolates identified as *S. pneumoniae* by conventional phenotypic methods during calendar year 2012 had been archived, and 100 of these isolates were randomly selected for the present study by choosing every second unique isolate recovered between January and November 2012. Most of the isolates had been recovered from respiratory or blood specimens, and had been identified prior to archiving as *S. pneumoniae* by examination of colonial and microscopic morphology, and optochin susceptibility testing in CO_2-enriched air. Also included in the present study was a convenience sample of 111 archived clinical isolates that had been identified prior to archiving as *S. mitis* based on conventional phenotypic methods, which included examination of colonial and microscopic morphology, and characterization using the API 20 Strep strip (bioMérieux). Between approximately 1995 and 1998, all *S. mitis* isolates that required full species identification for clinical purposes (most of which had been recovered from blood or deep tissue) were archived in our laboratory. For the present study, we selected the first 111 unique, viable isolates we could locate in the frozen archive.

Each of the 211 isolates included in this study was identified using the VITEK MS v2.0 system (bioMérieux, Marcy l'Etoile, France) after overnight growth on tryptic soy agar with 5% sheep blood (Remel, Lenexa, KS). Isolated bacterial colonies were applied (without prior extraction) to a single well of a disposable target slide, then overlaid with a matrix solution and air-dried prior to analysis, as described previously

[30]. If the VITEK MS method provided a split-identification or no identification, the isolate was re-analyzed once. If a single, species-level identification was provided upon repeat analysis, this identification was considered to be the final VITEK MS result; if a split identification or no identification was provided upon repeat analysis, no further analysis was performed.

The outcome of identification using the VITEK MS was compared with the original (pre-archiving) phenotypic identification (Table 1). When the VITEK MS identification matched the original phenotypic identification, no further testing was performed. When there were discrepancies (N=32), supplementary methods were applied to arrive at a definitive identification. These included bile solubility testing by the tube method; parallel optochin susceptibility testing in ambient and CO_2-enriched air; analysis using the VITEK 2 GP card (bioMérieux); application of the AccuProbe *Streptococcus pneumoniae* hybridization probe (Gen-Probe); and/or sequence analysis of the 16S rRNA gene [31, 32], *sodA* gene [33], *gro*EL gene [34] and/or *rec*A gene (Table 2) [35]. All gene sequences were edited using ChromasPro software (Technelysium, South Brisbane, Australia) and analyzed using NCBI BLASTn and leBIBI V5 [36]. Gene sequencing and analysis was performed by a scientist (CDG) at bioMérieux, who was blinded to the VITEK MS results. All other methods were performed by independent investigators at Massachusetts General Hospital. Using this approach, it was determined that the present study included 95 *S. pneumoniae* isolates and 116 non-pneumococcal isolates from within the *S. mitis* group (93 *S. mitis/oralis*, 12 *S. parasanguinis*, 2 *S. australis*, 2 probable *S. australis*, 3 *S. pseudopneumoniae*, 2 probable *S. infantis*, 1 *S. cristatus*, and 1 *S. sanguinis*).

Among 95 *S. pneumoniae* isolates, 94 (99%) were identified as *S. pneumoniae* by the VITEK MS v2.0 system; the remaining *S. pneumoniae* isolate was not identified by the VITEK MS (Table 1). Among 116 non-pneumococcal *S. mitis* group isolates, 102 (88%) were correctly identified to the species-level by the VITEK MS v2.0 system. Only one of these 116 isolates (<1%), a probable *S. infantis* isolate according to sequence analysis, was misidentified as *S. pneumoniae* (Table 2). Six additional non-pneumococcal isolates were assigned the correct genus but incorrect species by the VITEK MS v2.0 system; in each case, however, the incorrect identification placed the isolate within the *S. mitis* group and did not classify it as *S. pneumoniae* (Table 2). Seven non-pneumococcal isolates were assigned a split identification by the VITEK MS, but *S. pneumoniae* was never included among the alternatives (Table 2). Notably, 6 of the 7 misidentified isolates, and 2 of the 7 isolates assigned a split identification, could not be definitively identified by conventional phenotypic methods. Rather, these isolates required nucleic-acid sequence-based analysis of multiple gene targets for confident identification, demonstrating the challenging nature of these particular isolates. Also, 5 of the 7 misidentified isolates, and 1 of the 7 isolates assigned a split identification were *S. australis* or *S. infantis* isolates according to the results of DNA sequence analysis; these species are not represented in the VITEK MS v2.0 system database.

A limitation of this study is the fact that all clinical isolates were collected at a single site (Massachusetts General Hospital), and thus there was not broad geographic representation. However, the present study's findings are similar to those of a recent multi-center study in which the VITEK MS v2.0 system's performance was determined at 5 geographically diverse trial sites [30]. Compared with the multi-center study, the

present study included a larger number of *S. mitis* group clinical strains, none of which had been included in the multi-center study. A second limitation of the present study is the potential for selection bias. The non-pneumococcal isolates, unlike the *S. pneumoniae* isolates, were chosen by convenience rather than by a truly random selection process. And, although the *S. pneumoniae* isolates were chosen randomly and were unique isolates (only one isolate from an individual patient was included), it is possible that a clone (identical strain) could have been circulating among some of the patients from whom the isolates were derived. Finally, in this study we avoided performing a protein extraction step prior to analysis using the VITEK MS system, even when the VITEK MS provided no identification or a split identification. Although this was done in order to challenge the system in the most stringent fashion, the addition of an extraction step is known improve MALDI-TOF MS performance [37], and had it been applied it may have influenced our findings.

In summary, MALDI-TOF MS using the VITEK MS v2.0 system provides an accurate, fast, inexpensive and technically non-demanding means of discriminating between *S. pneumoniae* and other *S. mitis* group species. Adoption of this method in the clinical laboratory may improve the ability to make this clinically-relevant distinction.

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Table 1. Performance of the VITEK MS v2.0 system in distinguishing *S. pneumoniae* from non-pneumococcal *S. mitis* group species.

	Identification by Reference Methods	
	S. pneumoniae	Non-pneumococcal species
VITEK MS identification		
S. pneumoniae	94	1
Non-pneumococcal species	0	108
Split identification[a]	0	7
No identification	1	0
Total	95	116

[a]For these isolates, more than one possible identification was reported by the VITEK MS instrument.

312 Table 2. Resolution of discrepancies between original conventional identification and VITEK MS identification.

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Original conventional identification	VITEK MS identification	Identification based on reference methods	Reference methods applied	Number of isolates
S. pneumoniae	*S. pseudopneumoniae*	*S. pseudopneumoniae*	BS, Opt, HProbe, 16S, sodA, groEL[a]	3
S. pneumoniae	*S. mitis/oralis*	*S. mitis*	BS, HProbe, 16S, sodA[b]	2
S. mitis/oralis	*S. parasanguinis*	*S. australis*	16S, sodA	2
S. mitis/oralis	*S. parasanguinis*	Probable *S. australis*	16S, sodA, groEL, recA	2
S. mitis/oralis	*S. parasanguinis*	*S. parasanguinis*	VGP	12
S. mitis/oralis	*S. pneumoniae*	Probable *S. infantis*	BS, Opt, HProbe, 16S, sodA, groEL, recA	1
S. mitis/oralis	*S. cristatus*	*S. cristatus*	16S, sodA	1
S. mitis/oralis	*S. cristatus*	*S. mitis*	16S, sodA	1
S. mitis/oralis	*S. pseudopneumoniae*	*S. mitis*	BS, Opt, VGP, HProbe, 16S, sodA	1
S. mitis/oralis	Split: *S.mitis/oralis; S. parasanguinis*	Probable *S. infantis*	16S, sodA, groEL, recA	1
S. mitis/oralis	Split: *S. anginosus; Vibrio cholerae; Lactobacillus paracasei; Lactobacillus casei*	*S. mitis*	16S, sodA	1
S. mitis/oralis	Split: *S. mitis/oralis; S. sanguinis*	*S. mitis/oralis*	VGP	1
S. mitis/oralis	Split: *S. parasanguinis; Finegoldia magna*	*S. mitis/oralis*	VGP	1
S. mitis/oralis	Split: *Prevotella denticola; Parvimonas micra; S. parasanguinis*	*S. mitis/oralis*	VGP	1
S. mitis/oralis	Split: *S. mitis/oralis; S. intermedius*	*S. mitis/oralis*	VGP	1
S. mitis/oralis	Split: *S. parasanguinis; Bifidobacterium* sp.	*S. sanguinis*	VGP	1

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315 **Note:** BS, bile solubility testing using the tube method; Opt, optochin susceptibility testing in parallel using CO_2-enriched air and

316 ambient air; HProbe, AccuProbe *Streptococcus pneumoniae* DNA hybridization probe; 16S, DNA sequencing of the 16S rRNA gene;

317 sodA, DNA sequencing of the *sodA* gene; groEL, DNA sequencing of the *groEL* gene; VGP, VITEK 2 GP card; recA, DNA

318 sequencing of the *recA* gene.

319 [a] One of these 3 isolates was also analyzed by sequencing the *recA* gene.

320 [b] One of these 2 isolates was also analyzed by sequencing the groEL gene.

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