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Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Is a Sensitive and Specific Method for Identification of Aerococci

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Conventional methods for the identification of human-pathogenic aerococci to the species level are not reliable. We show that matrix-assisted laser desorption ionization–time of flight mass spectrometry correctly identifies aerococci to the species level and that it can be used to identify aerococci with high specificity in the diagnostic clinical microbiology laboratory.

Aerococci make up a genus of bacteria that are increasingly recognized as human pathogens. The two most clinically important aerococcal species are *Aerococcus urinae* and *Aerococcus sanguinicola*, which can cause urinary tract infections (1, 2), bacteremia (3, 4), and infective endocarditis (4–6). Aerococci are facultative anaerobic, Gram-positive, and catalase-negative bacteria. Colonies on blood agar resemble alpha-hemolytic streptococci, but on Gram staining, they appear as clusters like staphylococci. Consequently, misidentification of aerococci in the clinical microbiology laboratory commonly occurs (1, 7). Commercially available methods such as Vitek 2 and API-strep, as well as colony morphology and biochemical tests, are unable to safely separate aerococci from other bacteria or differentiate aerococcal species from each other (1, 8). Secure identification of aerococci has relied on sequencing of the 16S rRNA gene. A newly introduced method for identification to the species level in clinical microbiology is matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS). It has been shown to be a reliable and fast method for the identification of commonly isolated bacteria in the clinical microbiological laboratory (9, 10). These studies, in which bacterial strains were prospectively collected from clinical samples, indicate that MALDI-TOF MS has the potential to replace conventional identification techniques. However, the accuracy of MALDI-TOF MS identification of bacterial species that are uncommon in clinical samples, such as aerococci, needs to be further evaluated. In a recent study, a collection of well-characterized catalase-negative, Gram-positive cocci including 35 aerococcal strains (of which 27 were *A. urinae*, 5 were *A. sanguinicola*, 2 were *Aerococcus viridans*, and 1 was *Aerococcus christensenii*) was subjected to MALDI-TOF MS analysis (11). Analysis of each isolate was performed first with Biotyper version 2.0 and then with an extension of the database. All of the *A. urinae* and *A. sanguinicola* isolates were correctly identified to the species level, and high scores were obtained especially with an extended database. Herein we evaluate the usefulness of MALDI-TOF MS for the correct identification of *A. urinae* and *A. sanguinicola* in a clinical setting by defining both the sensitivity and the specificity of the method.

To define the sensitivity of the method, expressed as the proportion of actual aerococci that are correctly identified, all urine isolates at the two routine diagnostic laboratories of clinical microbiology in Malmö and Lund, University and Regional Laboratories, Region Skåne, Sweden, were prospectively screened for the presence of aerococci during a 2-month period in 2010. Colonies from all plates with at least 10^3 colonies (10^8 CFU/liter) resembling alpha-hemolytic streptococci were tested for catalase activity and Gram stained. All catalase-negative isolates that were not obviously microscopically characterized as Gram-positive cocci in chains were subjected to 16S rRNA sequencing as previously described (12). Of 48 isolates that were subjected to sequencing, 22 were *A. urinae* and 19 were *A. sanguinicola*; no other aerococcal species were observed. The nonaerococcal isolates were *Gemella*

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TABLE 1 Sensitivity of MALDI-TOF MS in the identification of *A. urinae* and *A. sanguinicola*

Species (n)[a] according to:				
16S rRNA gene sequencing	MALDI-TOF MS	Median score (range)[b]	Species (n)[a] according to Vitek 2	Median % probability (range)
A. urinae (22)	*A. urinae* (22)	2.25 (2.08–2.39)	*A. urinae* (10)	94 (87–99)
			Granulicatella adiacens (5)	96 (91–98)
			Low discrimination[b] (5), unidentified (2)	
A. sanguinicola (19)	*A. sanguinicola* (19)	2.25 (2.08–2.39)	*A. viridans* (18)	95 (86–97)
			Unidentified (1)	

[a] n, number of isolates.
[b] Gives the same probability for more than one species.

TABLE 2 Specificity of MALDI-TOF MS in the identification of *A. urinae* and *A. sanguinicola*

Species (*n*)[a] according to MALDI-TOF MS	Median score (range)	Species (*n*)[a] according to 16S rRNA gene sequencing
A. urinae (25)	2.19 (1.90–2.48)	*A. urinae* (25)
A. sanguinicola (19)	2.28 (1.83–2.49)	*A. sanguinicola* (19)

[a] *n*, number of isolates.

morbillorum ($n = 1$), *Enterococcus durans/faecium* ($n = 1$), *Actinobaculum schalii* ($n = 1$), *Streptococcus mitis* ($n = 2$), *Streptococcus anginosus* ($n = 1$), and a staphylococcal species ($n = 1$). The aerococcal isolates were prepared by the direct-transfer method (direct smear) (9) and subjected to analysis by Ultraflextreme MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) with the Biotyper version 3.0 software without modifications. The results for all 41 strains were identical to those obtained from the sequencing of the 16S rRNA gene. For two *A. urinae* isolates that grew poorly, a score of <2.00 (the recommended cutoff for probable identification to the species level according to the manufacturer) was obtained with the Biotyper software after MALDI-TOF MS analysis and these isolates were subjected to a protein extraction method as described previously (9), after which a score of >2.00 was acquired. The range of scores obtained by the Biotyper software after MS analysis was 2.08 to 2.39 (median, 2.25) for *A. urinae* and 2.09 to 2.61 (median, 2.37) for *A. sanguinicola*. The same isolates were also tested with the Vitek 2 system (bioMérieux, Marcy, l'Etoile, France), which classified 18 of the *A. sanguinicola* isolates as *A. viridans* and failed to identify 12 of the *A. urinae* isolates (Table 1). We conclude that MALDI-TOF MS correctly identifies *A. urinae* and *A. sanguinicola* to the species level and that the method has excellent sensitivity in this respect.

We proceeded by implementing MALDI-TOF MS in the standard diagnostic procedures for suspected aerococci. All colonies from urine cultures with the appearance of alpha-hemolytic streptococci ($\geq 10^8$ CFU/liter and no more than one other species in the culture) were subjected to MALDI-TOF MS. During a 2-month period in 2012, isolates that were identified as *A. urinae* ($n = 25$) or *A. sanguinicola* ($n = 19$) (scores of ≥1.80) were subjected to 16S rRNA gene sequencing. Thus, the specificity of MALDI-TOF MS in the identification of aerococci to the species level could be evaluated. The range of scores obtained with the Biotyper software was 1.90 to 2.48 for *A. urinae* and 1.83 to 2.49 for *A. sanguinicola*. The median score for *A. urinae* was 2.19, and that for *A. sanguinicola* was 2.28. There was complete agreement between the results of the MALDI-TOF MS analysis and the sequencing of the 16S rRNA gene for all 44 isolates, demonstrating that MALDI-TOF MS is also specific in identifying aerococci to the species level in the standard diagnostic clinical microbiology laboratory (Table 2).

Difficulties in identifying aerococci have most likely led to an underestimation of the incidence of infections with these bacteria and also of the clinical importance of this genus, particularly in the case of *A. sanguinicola*. Many case reports on *A. viridans* infections have been published on the basis of identification to the species level using Vitek 2 or API-strep (8, 13, 14), and most of these are likely to have been *A. sanguinicola*, which is far more common than *A. viridans* in our laboratory. Importantly, resistance to fluoroquinolones seems to be more frequent in *A. sanguinicola* isolates than in *A. urinae* isolates (2, 15, 16), making it clinically relevant to differentiate between these aerococcal species. In conclusion, we demonstrate that MALDI-TOF MS is a reliable method for identification of aerococci to the species level. We suggest that this method should be employed for aerococcal identification in clinical microbiology laboratories where the technique is available.

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