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Comparison of the Accuracy of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry with That of Other Commercial Identification Systems for Identifying *Staphylococcus saprophyticus* in Urine

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Among 30 urinary isolates of *Staphylococcus saprophyticus* identified by sequencing methods, the rate of accurate identification was 100% for Bruker Biotyper matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), 86.7% for the Phoenix PID and Vitek 2 GP systems, 93.3% for the MicroScan GP33 system, and 46.7% for the BBL CHROMagar Orientation system.

Staphylococcus saprophyticus, a coagulase-negative staphylococcus (CoNS), is a common pathogen associated with community-acquired uncomplicated urinary tract infections (UTIs) in humans, particularly women younger than 50 years of age (1). Of the urinary CoNS isolates, *S. saprophyticus* is identified presumptively by simplified conventional methods and commercially automated identification methods (2–9). However, misidentification of *S. saprophyticus* by these simplified conventional and automated phenotypic methods often occurs. Although matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has been used for many years in the fields of proteomics and toxicology, the technology has only recently been applied to the field of microbiology for species identification of bacteria (2, 10–16). Direct detection of urinary tract pathogens from urine samples with high accuracy by MALDI-TOF MS has been reported, especially for Gram-negative bacteria with high bacterial counts (17).

In this study, we evaluated a total of 34 presumptive isolates of *S. saprophyticus* obtained from the urine of patients with UTIs at the National Taiwan University Hospital during the period January 2004 to December 2011. The 34 isolates were presumptively identified as *S. saprophyticus* based on colonial and microscopic morphotypes characteristic of the organism, the absence of hemolytic activity or coagulase production, resistance to novobiocin (5-μg disk; no inhibition zone), and susceptibility to polymyxin (300-IU disk; inhibition zone of ≥10 mm). Identification of the 34 isolates to the species level was performed using the BBL CHROMagar Orientation system (Becton Dickinson, Diagnostic Systems, Sparks, MD) and three commercially automated identification systems, namely, the Phoenix PMIC/ID-30 automated system (Becton Dickinson), the Vitek 2 GP system (bioMérieux Inc., La Balme les Grottes, France), and the MicroScan Walkaway automated system (Dade Behring, Inc., West Sacramento, CA). *S. saprophyticus* ATCC 15305 was used as a quality control strain for the BBL CHROMagar Orientation system.

For performance of the Bruker Biotyper MALDI-TOF MS system (Bruker Daltonics), a single colony from a blood agar plate was subjected to an ethanol-formic acid extraction procedure for microorganism profiling (4). Spectra were analyzed using MALDI Biotyper automation control and the Bruker Biotyper 3.1 software and library (version 3.1.66, with 4,613 entries; Bruker Daltonics). Identification scores of ≥2.000 indicated species-level identification, scores of 1.700 to 1.999 indicated genus-level identification, and scores of <1.700 indicated no identification (4). Cluster analysis of the isolates was performed using ClinProtools 3.0 (Bruker Daltonics).

PCR amplification of the nearly complete 16S rRNA gene (1,463 bp) from the 34 isolates was performed with two primers (8FPL and 1492) as previously described (18). The sequences obtained were compared with published sequences in the GenBank database, using the BLASTN algorithm (http://www.ncbi.nlm.nih.gov/blast). PCR amplification and sequencing of the partial *rpoB* gene of the 34 isolates were performed using primers 2491F and 3241R (16, 19, 20).

Of the 34 isolates, 30 were identified as *S. saprophyticus*, 2 were identified as *S. cohnii*, 1 was identified as *S. xylosus*, and 1 was identified as *S. sciuri* by 16S rRNA and *rpoB* gene sequencing. The 30 isolates of *S. saprophyticus* were recovered from 30 voided urine samples from 24 patients, including 23 women and 1 man, with a mean age of 36 years (range, 19 to 63 years). Six female patients had two different urine samples that were positive for *S. saprophyticus*. Of the 30 isolates that had been identified by sequencing as being *S. saprophyticus*, 22 (73.3%) were correctly identified as *S. saprophyticus* by the Phoenix PMIC/ID-30 system (confidence values, 90 to 99%), the Vitek 2 GP system (probability of identification, 99%), and the MicroScan system (99.4 to 99.9% identification). The Phoenix PID system misidentified 13.3% (4/30 isolates) of the isolates, the Vitek 2 GP system resulted in

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TABLE 1 Differences among various microbial identification methods in identifying 12 urinary CoNS isolates that were presumptively identified as *S. saprophyticus*

Isolate no.	Identification result					Colony color on BBL CHROMagar Orientation agar/class
	Sequencing of 16S rRNA and *rpoB* genes	MALDI-TOF Biotyper (score/cluster)	Vitek II GP (probability of identification [%])	Phoenix PID (confidence value [%])	MicroScan GP33 (% identification)	
1	*S. saprophyticus*	*S. saprophyticus* (2.11/B)	*S. saprophyticus* (99)	*S. saprophyticus* (90)	*S. hominis* (72.7)	White/II
2	*S. saprophyticus*	*S. saprophyticus* (2.044/B)	*S. saprophyticus* (99)	*S. epidermidis* (90)	*S. saprophyticus* (99.7)	White/II
3	*S. saprophyticus*	*S. saprophyticus* (2.007/B)	*S. saprophyticus* (99)	*S. epidermidis* (90)	*S. saprophyticus* (99.9)	White/II
4	*S. saprophyticus*	*S. saprophyticus* (2.120/B)	*S. saprophyticus* (99)	*S. warneri* (90)	*S. saprophyticus* (86.0)	White/II
5	*S. saprophyticus*	*S. saprophyticus* (2.121/B)	*S. hominis* (96)	*S. saprophyticus* (94)	*S. schleiferi* (71)	White/II
6	*S. saprophyticus*	*S. saprophyticus* (2.154/B)	*S. warneri* (94)	*S. saprophyticus* (99)	*S. saprophyticus* (99.7)	White/II
7	*S. saprophyticus*	*S. saprophyticus* (1.993/B)	*S. warneri* or *S. hominis*	*S. saprophyticus* (99)	*S. saprophyticus* (99.7)	White/II
8	*S. saprophyticus*	*S. saprophyticus* (2.103/A)	*S. hominis* (low)	*S. epidermidis* (92)	*S. saprophyticus* (99.9)	Pink/I
9	*S. cohnii*	*S. cohnii* (1.495)	*S. xylosus* (95)	*S. equorum* (99)	*S. xylosus* (99.2)	Blue
10	*S. cohnii*	*S. succinus* (1.394)	*S. xylosus* (95)	*S. equorum* (99)	*S. xylosus* (97.3)	Blue
11	*S. xylosus*	*S. xylosus* (2.073)	*S. xylosus* (98)	*S. gallinarum* (97)	*S. xylosus* (90.1)	White
12	*S. sciuri*	*S. sciuri* (2.036)	*S. sciuri* (99)	*S. sciuri* (99)	*S. sciuri* (99.9)	Blue

misidentification of an equal number of isolates (13.3% [4/30 isolates]), and the MicroScan system misidentified 6.7% (2/30 isolates) of the isolates (Table 1). Figure 1 shows the colony characteristics of two *S. saprophyticus* isolates and of one isolate each of *S. cohnii*, *S. xylosus*, and *S. sciuri* on BBL CHROMagar Orientation agar. Among the 30 isolates of *S. saprophyticus*, 14 (46.7%; class I) yielded pink/opaque colonies that are characteristic of *S. saprophyticus* on BBL CHROMagar Orientation agar, and 16 (53.3%; class II) showed white colonies.

MALDI-TOF MS profiles of the five different CoNS species are illustrated in Fig. 2A. About 10 to 20 prominent ion peaks were noted in the *m/z* 2,000 to 12,000 range. Of the two isolates of *S. cohnii* that had been identified by genetic methods, the Biotyper MALDI-TOF system identified one as *S. cohnii* and one as *S. succinus* (Table 1); however, the identification scores were low. Of the 30 isolates of *S. saprophyticus*, 23 (76.7%) had identification scores of >2.0 (ranging from 2.005 to 2.154), and 7 (23.3%) had identification scores ranging from 1.893 to 1.997.

Cluster analysis of the 30 isolates was performed based on the colonial characteristics on CHROMagar Orientation agar (pink/opaque colonies for class I and white colonies for class II). Ten spectra for each class were selected randomly for model genera-

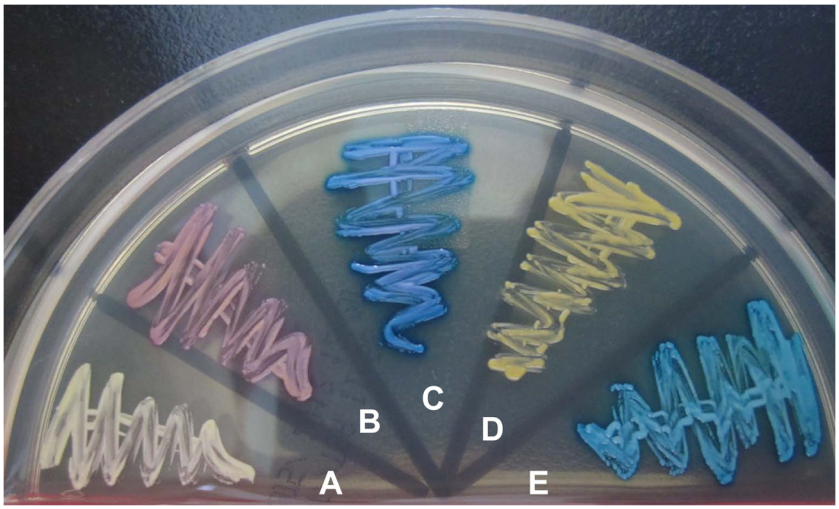

FIG 1 Colonial characteristics on CHROMagar Orientation agar of four different *Staphylococcus* species that were identified by 16S rRNA gene sequencing and a Biotyper MALDI-TOF MS system. Isolates: A and B, *S. saprophyticus*; C, *S. cohnii*; D, *S. xylosus*; E, *S. sciuri*.

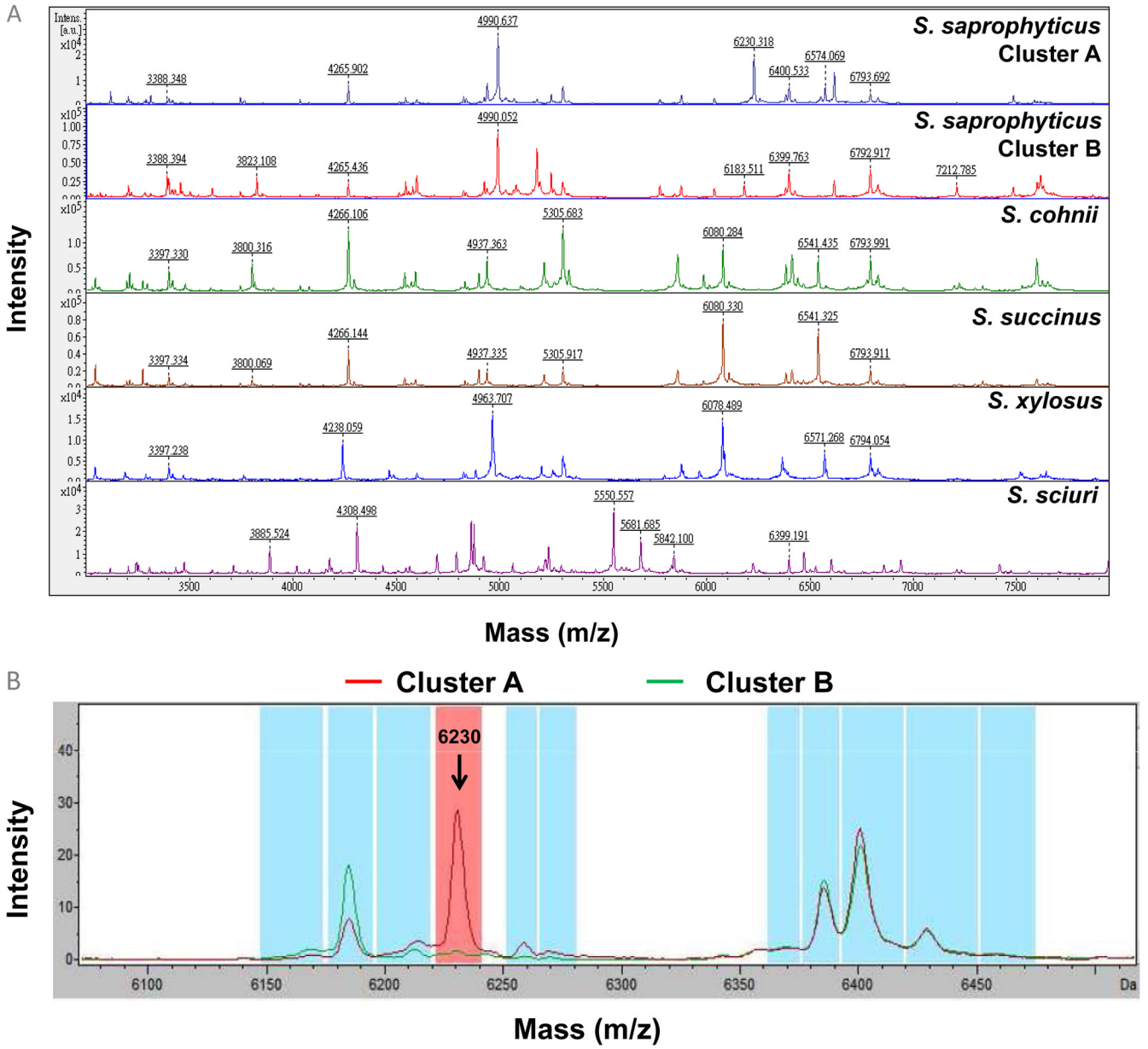

FIG 2 (A) MALDI-TOF MS identification of *Staphylococcus saprophyticus* class I (pink colony growth on CHROMagar Orientation agar), *S. saprophyticus* class II (white colony growth on CHROMagar Orientation agar), *S. cohnii*, *S. succinus*, *S. xylosus*, and *S. sciuri.* The absolute intensities of the ions are shown on the *y* axis, and the masses (*m/z*) of the ions are shown on the *x* axis. The *m/z* value represents the mass-to-charge ratio. (B) Two clusters of *S. saprophyticus* spectra, i.e., cluster A and cluster B, analyzed by clustering analysis of MALDI-TOF results. The cluster A average spectrum is demonstrated in red, and the cluster B average spectrum is demonstrated in green. The arrow shows the peak 6,230 *m/z* signal. The 6,230 *m/z* signal could be observed in the cluster A spectrum but not the cluster B spectrum.

tion by ClinProtools 3.0, using the QuickClassifier algorithm (Bruker Daltonics). This model included eight peaks (3,115, 5,068, 6,213, 6,230, 6,258, 6,928, 8,223, and 9,926 *m/z*) for classification. All 30 *S. saprophyticus* isolates were classified using this model, and the results revealed that a cluster A spectrum was present for 13 (92.8%) of 14 class I isolates, with a cluster B spectrum for 16 (100%) of 16 class II isolates (Fig. 2B). A characteristic 6,230 *m/z* signal was observed in the cluster A spectrum but not the cluster B spectrum (Fig. 2B). Isolates from the same patients belonged to the same clusters. There was no clinical association of patients (underlying medical conditions or types of UTIs) with isolates belonging to the two clusters. This clustering of *S. saprophyticus* isolates was not observed previously using MALDI-TOF or other commercial methods.

Several factors are associated with *S. saprophyticus* UTIs, including outdoor swimming, sexual intercourse, and work in meat and cheese production (1). In contrast, UTIs caused by other CoNS, particularly *S. epidermidis*, are commonly related to indwelling catheters or surgery and cause complicated hospital-acquired UTIs (1). As a result, accurate identification of CoNS to the

species level, or at least rapid differentiation between *S. saprophyticus* and other CoNS isolates, is important.

In the present study, three important findings were illustrated. First, the accuracy of identification of *S. saprophyticus* by use of the recommended presumptive identification scheme for *S. saprophyticus* among urinary CoNS isolates was better than that using the BBL CHROMagar Orientation system (88.2% versus 46.7%). Second, among the 30 isolates genetically identified as *S. saprophyticus*, misidentification was found more commonly using the Phoenix PID and Vitek 2 GP systems than using the MicroScan system. Third, all 30 of the *S. saprophyticus* isolates were accurately identified by the Biotyper MALDI-TOF system. A previous study demonstrated that MALDI-TOF MS could accurately identify all CoNS species and subspecies tested (16). However, one isolate of *S. cohnii* in this study could not be identified accurately to the species level by MALDI-TOF MS.

Few studies have compared the accuracies of identifying *S. saprophyticus* in urine of the Vitek 2, Phoenix, and MicroScan systems (2, 5, 8). The Vitek 2 system has been shown to be better than the Phoenix system at identifying CoNS isolates to the species level (91.9% versus 88.4%) when molecular reference methods are used (2). In addition, the MicroScan system has been reported to have an accuracy rate of 90% for correctly identifying *S. saprophyticus* in urine (5). Only a small number of *S. saprophyticus* isolates have been tested in previous studies for assessment of the identification accuracy of the BBL CHROMagar Orientation system (3, 6). The results of our study clearly demonstrate that two distinct colony colors (white and pink) for *S. saprophyticus* on BBL CHROMagar Orientation agar were not in accordance with the interpretation instructions provided by the manufacturer.

In summary, our findings show that MALDI-TOF MS is more accurate than other commercial identification methods for identifying CoNS, particularly urinary isolates of *S. saprophyticus*.

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