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Comparison of a New Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Platform, ASTA MicroIDSys, With Bruker Biotyper for Species Identification

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Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, with its accuracy and speed, is widely used for bacterial identification. The ASTA MicroIDSys system (ASTA, Korea) was recently developed for species identification. We compared its performance with that of Bruker Biotyper (Bruker Daltonics, Germany). Microbes were recovered from sputum, urine, and pus samples from patients admitted to a tertiary care hospital in Korea from January to April 2016. Matrix solution (α-cyano-4-hydroxycinnamic acid) was used, and the peptide profiles acquired from the Microflex LT (Bruker Daltonics) and Tinkerbell LT (ASTA) were analyzed by using their respective software. From 5,322 isolates, Bruker Biotyper identified 163 species; fifty species from 4,919 isolates were identified more than 10 times, including *Klebsiella pneumoniae* (n=571), *Acinetobacter baumannii* (n=436), *Pseudomonas aeruginosa* (n=358), *Escherichia coli* (n=372), *Staphylococcus aureus* (n=511), *S. epidermidis* (n=444), *Enterococcus faecium* (n=262), *E. faecalis* (n=220), and *Candida albicans* (n=248). Identical results, confidence scores (≥ 2.0 for Bruker Biotyper), and acceptable scores (≥140 for ASTA MicroIDSys) were obtained for 86.1% of isolates. Of 4,267 isolates, 99.2% showed acceptable scores in both systems. Results from the ASTA MicroIDSys showed good agreement with those from the Bruker Biotyper. The ASTA MicroIDSys could reliably identify clinically important microorganisms.

Key Words: MicroIDSys, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, Bruker Biotyper

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Bacterial identification with automated instruments or conventional methods such as biochemical reactions takes a few hours to days in clinical microbiology laboratories. More rapid methods are necessary to diagnose and treat septic patients, and better accuracy is necessary for classifying complicated bacterial mixtures. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is widely used for bacterial identification in clinical microbiology laboratories because of its speed and accuracy [1-3].

Two in vitro diagnostic MALDI-TOF MS systems, the Bruker Biotyper MS (Bruker Daltonics, Bremen, Germany) and the Vitek MS (bioMérieux, Marcy l'Etoile, France), have been implemented in clinical microbiology laboratories worldwide and are routinely used for identifying bacterial and yeast isolates [4-6].

Recently, a new system, the ASTA MicroIDSys system (ASTA, Suwon, Korea), was developed for identification of clinically important pathogenic species. The ASTA MicroIDSys system consists of a linear-type MALDI-TOF MS, a database, and software for species identification by spectral pattern matching. The linear-type MALDI-TOF MS performs microbial MS analysis in the range of m/z=2,000–20,000, with a mass accuracy and resolving power of 250 ppm and 1,000, respectively. The database contains reference MALDI spectra for 2,604 species. The MicroIDSys software employs an auto-selection algorithm for mass peaking of each species or strain of microorganism, in which the number of peaks that specifies each species is selected by the machine, based on pre-determined parameters, for better accuracy. The machine program itself selects parameters and masses as well as intensities of importance. In the present study, we compared the performance of the ASTA MicroIDSys system with that of the Bruker Biotyper MS system for identifying bacteria and yeast in routine clinical microbiology laboratory for the first time.

A total of 5,322 isolates were recovered from clinical specimens of urine, sputum, tracheal aspirate, wounds, and pus from patients admitted to a tertiary care hospital in Korea in January-April 2016. The specimens were inoculated in appropriate media such as 5% sheep blood agar, MacConkey agar, or chocolate agar for bacteria and Sabouraud dextrose agar for yeast, and then incubated for 24–48 hr at 35°C. A single bacterial colony from the agar was smeared onto the target plate (Bruker Daltonics GmbH), the matrix solution (α-cyano-4-hydroxycinnamic acid) was overlaid on the spot, and the peptide profile was acquired from the Bruker Microflex LT system. For yeast analysis, suspicious colonies were smeared directly onto the target plate and overlaid with 1 μL 70% formic acid (Sigma-Aldrich, St. Louis, MO, USA) and matrix solution. The Microflex system had the Biotyper software 3.1 and the MALDI Biotyper reference library version 5.0.0.0. The mass spectra were analyzed according to the manufacturer's instructions. We used identification score values ≥2.0 for bacteria and yeast. After complete analysis using the Bruker Biotyper, the peptide profiles were obtained by using the ASTA MicroIDSys on the same target plates. All the mass profiles were then analyzed by using the MicroIDSys 1.0. The cut-off value was set at ≥140 for the ASTA MicroIDSys for all microorganisms. PCR and 16S rRNA gene sequencing were performed for isolates that showed different results from the Bruker Biotyper and ASTA MicroIDSys systems.

Among the 5,322 isolates, 50 species (from 4,919 isolates) were isolated more than 10 times and analyzed for comparing the performances of the two MALDI-TOF MS systems. The results were as follows: 2,222 gram-negative bacilli, 1,926 gram-positive cocci, 413 *Candida* spp., and 385 other bacteria were detected. The most frequently isolated bacteria were *Klebsiella pneumoniae* (n=571), followed by *Acinetobacter baumannii* (n=436), *Pseudomonas aeruginosa* (n=358), *Escherichia coli* (n=372), *Staphylococcus aureus* (n=511), *S. epidermidis* (n=444), *Enterococcus faecium* (n=262), *E. faecalis* (n=220), *Corynebacterium striatum* (n=201), and *Candida albicans* (n=248).

From the 4,919 isolates studied, identical results with confidence scores (≥2.0 for the Bruker Biotyper MS system) and acceptable scores (≥140 for the ASTA MicroIDSys system) were obtained for 4,234 (86.1%) isolates (Table 1). For the bacteria that are frequently isolated in clinical microbiology laboratories, the high agreement rates were as follows: *K. pneumonia* (100%), *E. coli* (98.9%), *P. aeruginosa* (100%), *A. baumannii* (99.8%), *S. aureus* (99.8%), *S. epidermidis* (99.8%), *E. faecium* (98.9%), and *E. faecalis* (99.1%). In addition, 4,841 (98.4%) isolates had a Bruker Biotyper score ≥1.7 and an ASTA MicroIDSys score ≥140. Only 78 (1.6%) isolates showed discrepant results between the two systems. For these isolates, we performed 16S rRNA gene sequencing. However, some species in the isolates were not accurately identified by either of the two methods; the 16S rRNA gene sequence similarity was very high for *Enterobacter* and *Streptococcus mitis* groups [7].

From the observed discrepant results between the two MALDI-TOF MS systems, we suspected that a known limitation of other MALDI-TOF MS systems might also be present in the ASTA MicroIDSys. Microorganisms are identified by MALDI-TOF MS systems using prerecorded protein spectra that are present in the system library, and these spectra are mostly based on ribosomal proteins. Therefore, MALDI-TOF MS systems are intrinsically limited to differentiate closely related species or strains of *Salmonella* spp., *Raoutella, Klebsiella, Enterobacter*, and *Citrobacter* [4, 8].

Identical results, with scores between 1.7 and 2.0 for the Bruker Biotyper MS system and acceptable scores ≥140 for the ASTA MicroIDSys system, were obtained for 581 (11.8%) isolates; these included 242 (58.6%) *Candida* spp., 205 (10.6%) gram-positive cocci, and 82 (3.7%) gram-negative bacilli. Only two isolates of *C. albicans* showed discrepant results with an ASTA MicroIDSys score <140. This result suggested that the threshold score for identification with the Bruker Biotyper should be 1.7, instead of the usual 2.0, in order to compensate for the spectrum quality of *Candida* spp. In contrast, the cutoff score used for identification by the ASTA MicroIDSys was the typical

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Table 1. Comparison of the results for frequently isolated bacteria from the Bruker Biotyper and ASTA MicroIDSys systems

Species	N of isolates with identical/discrepant results				
	Score (Bruker): ≥2.0		Score (Bruker): 1.7≤, <2.0		Total
	Score (ASTA):		Score (ASTA):		
	≥140	<140	≥140	<140	
Gram-negative bacilli					
Acinetobacter baumannii	420/1	3	12		435/1
Acinetobacter nosocomialis	18				18/0
Burkholderia cenocepacia	13				13/0
Citrobacter freundii	16	0/1	5		21/1
Elizabethkingia meningoseptica	11		2		13/0
Enterobacter aerogenes	75	0/1	1		76/1
Enterobacter asburiae	6/9		0/1		6/10
Enterobacter cloacae	42/3	1	3/1		46/4
Enterobacter kobei	13/4		0/1		13/5
Escherichia coli	360/1	0/2	8	0/1	368/4
Haemophilus influenzae	22	1		0/1	23/1
Klebsiella oxytoca	14	1			15/0
Klebsiella pneumoniae	539	1	31		571/0
Moraxella catarrhalis	19	1			20/0
Morganella morganii	25				25/0
Proteus mirabilis	26				26/0
Providencia rettgeri	14	1			15/0
Pseudomonas aeruginosa	349	1	8		358/0
Serratia marcescens	24		1		25/0
Stenotrophomonas maltophilia	96	1	11		108/0
N of subtotal (%)	2,102/18 (94.6/0.8)	11/4 (0.5/0.2)	82/3 (3.7/0.1)	0/2 (0/0.1)	2,195/27 (98.8/1.2)
Gram-positive cocci					
Enterococcus avium	12				12/0
Enterococcus faecalis	204	1/1	10/1	1	218/2
Enterococcus faecium	250/2	0/1	6		259/3
Enterococcus raffinosus	7		5		12/0
Staphylococcus aureus	501/1	1	7		510/1
Staphylococcus capitis	16		8		24/0
Staphylococcus epidermidis	356		84/1	2	443/1
Staphylococcus haemolyticus	81	0/1	42/1		125/2
Staphylococcus hominis	14		7		21/0
Staphylococcus lugdunensis	8		1/1		10/1
Streptococcus agalactiae	41		3		44/0
Streptococcus anginosus	59	1	3		63/0
Streptococcus constellatus	14		1/1		16/1

(Continued to the next page)

Table 1. Continued

Species	N of isolates with identical/discrepant results				
	Score (Bruker): ≥2.0		Score (Bruker): 1.7≤, <2.0		Total
	Score (ASTA):		Score (ASTA):		
	≥140	<140	≥140	<140	
Streptococcus mitis	20/1	0/1	3/1	0/1	27/4
Streptococcus oralis	30	1/2	4/3	2/4	46/9
Streptococcus parasanguinis	21		7		28/0
Streptococcus pneumoniae	14/10	2/2	4/3		35/15
Streptococcus salivarius	23		10		33/0
N of subtotal (%)	1,671/14	6/8	205/12	5/5	1,887/39
	(86.8/0.7)	(0.3/0.4)	(10.6/0.6)	(0.3/0.3)	(98.0/2.0)
Other bacteria					
Clostridium difficile	30		3	1	34
Clostridium hathewayi	13		1		14
Corynebacterium amycolatum	18				18
Corynebacterium striatum	190/1	0/4	6		196/5
Lactobacillus crispatus	8	1	13		22
Neisseria flavescens	11		2		13
Rothia mucilaginosa	28		27	1	56
N of subtotal (%)	298/1	1/4	52/0	2/0	353/5
	(83.2/0.3)	(0.3/1.1)	(14.5/0)	(0.6/0)	(98.6/1.4)
Candida spp.					
Candida albicans	93	0/1	149/3	0/2	242/6
Candida glabrata	27		21		48
Candida krusei	11		3	1	15
Candida parapsilosis	4		19		23
Candida tropicalis	28		50	0/1	79
N of subtotal (%)	163/0	0/1	242/3	1/3	406/7
	(39.5/0)	(0/0.2)	(58.6/0.7)	(0.2/0.7)	(98.3/1.7)
Total (%)	4,234/33	18/17	581/18	8/10	4,841/78
	(86.1/0.7)	(0.4/0.3)	(11.8/0.4)	(0.2/0.2)	(98.4/1.6)

140 value itself, indicating that the power to discriminate between *Candida* species was higher in the ASTA MicroIDSys system than in the Bruker Biotyper system. The ASTA MicroIDSys MS system also showed high accuracy rates for overall identification of bacteria and *Candida* spp. from isolates.

In this study, we identified clinically relevant bacteria and *Candida* species from clinical specimens using the ASTA MicroIDSys system. Our findings on ASTA MicroIDSys system performance in the identification of bacteria and *Candida* species are in high agreement with findings from the Bruker Biotyper system. Especially for frequently isolated bacteria, such as *K. pneumoniae, E. coli, P. aeruginosa, A. baumannii, S. aureus, S. epidermidis, E. faecium*, and *E. faecalis*, high agreement rates (98.9–100%) were shown. In conclusion, the ASTA MicroIDSys has comparable identification capability to the Bruker Biotyper system. The ASTA MicroIDSys system can reliably identify microorganisms that are commonly isolated in clinical microbiological laboratories.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Supplemental Data Table S1. Comparison of the identified bacteria and yeast with score values ≥2.0 for the Bruker Biotyper system and ≥140 for the ASTA MicroIDSys system

Species	N of isolates with:		Agreement rate (%)
	Identical results	Discrepant results	
Gram-negative bacilli			
Acinetobacter baumannii	420	1	99.8
Acinetobacter nosocomialis	18		100.0
Burkholderia cenocepacia	13		100.0
Citrobacter freundii	16		100.0
Elizabethkingia meningoseptica	11		100.0
Enterobacter aerogenes	75		100.0
Enterobacter asburiae	6	9	40.0
Enterobacter cloacae	42	3	93.3
Enterobacter kobei	13	4	76.5
Escherichia coli	360	1	99.7
Haemophilus influenzae	22		100.0
Klebsiella oxytoca	14		100.0
Klebsiella pneumoniae	539		100.0
Moraxella catarrhalis	19		100.0
Morganella morganii	25		100.0
Proteus mirabilis	26		100.0
Providencia rettgeri	14		100.0
Pseudomonas aeruginosa	349		100.0
Serratia marcescens	24		100.0
Stenotrophomonas maltophilia	96		100.0
N of subtotal	2,102	18	99.2
Gram-positive cocci			
Enterococcus avium	12		100.0
Enterococcus faecalis	204		100.0
Enterococcus faecium	250	2	99.2
Enterococcus raffinosus	7		100.0
Staphylococcus aureus	501	1	99.8
Staphylococcus capitis	16		100.0
Staphylococcus epidermidis	356		100.0
Staphylococcus haemolyticus	81		100.0
Staphylococcus hominis	14		100.0
Staphylococcus lugdunensis	8		100.0
Streptococcus agalactiae	41		100.0
Streptococcus anginosus	59		100.0
Streptococcus constellatus	14		100.0
Streptococcus mitis	20	1	95.2
Streptococcus oralis	30		100.0
Streptococcus parasanguinis	21		100.0
Streptococcus pneumoniae	14	10	58.3
Streptococcus salivarius	23		100.0
N of subtotal	1,671	14	99.2
Other bacteria			
Clostridium difficile	30		100.0
Clostridium hathewayi	13		100.0
Corynebacterium amycolatum	18		100.0
Corynebacterium striatum	190	1	99.5
Lactobacillus crispatus	8		100.0
Neisseria flavescens	11		100.0
Rothia mucilaginosa	28		100.0
N of subtotal	298	1	99.7
Candida spp.			
Candida albicans	93		100
Candida glabrata	27		100
Candida krusei	11		100
Candida parapsilosis	4		100
Candida tropicalis	28		100
N of subtotal	163		100
Total	4,234	33	99.2

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