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更新封面

Evaluation of the Performance of ASTA MicroIDSys, a Novel Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry System, in Identification of Bacterial Clinical Isolates

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Abstract

Background: We evaluated the performance of ASTA MicroIDSys (ASTA, Korea) and Bruker Biotyper (Bruker Daltonics, Germany) systems in the identification of bacterial isolates from clinical microbiology laboratory specimens during the study period. In addition, species for which the identification accuracy using MALDI-TOF MS systems were previously reported to be poor were also identified by comparing the MS results with those obtained using molecular identification. *Methods:* A total of 889 non-duplicated clinical isolates were included in this study. The results of ASTA MicroIDSys were compared with those of Bruker Biotyper; 16S rRNA sequencing was performed for the species for which results obtained using the two systems did not match. The sequences of *rpoB*, *hisA*, and/or *recA* for the clinical isolates of *Acinetobacter* species, *Klebsiella* species, and *Burkholderia cepacia* complex were analyzed and used as reference identifications. *Results:* The concordance rates for bacterial identification using ASTA MicroIDSys and Bruker Biotyper were 100% at the genus level and 98.3% at the species level for isolates belonging to the order *Enterobacterales*. Similarly, the concordance rates at the genus and species levels were 98.8% and 91.0% for glucose non-fermenting bacilli, 100% and 100% for gram-positive cocci, and 98.9% and 98.9% for other isolates, respectively. ASTA MicroIDSys was expected to correctly identify 97.9% of the 108,251 isolates identified in our clinical microbiology laboratory over the past 5 years. *Conclusion:* ASTA MicroIDSys showed excellent performance in bacterial identification for most of the clinically relevant species. Further extension of the database could improve the identification accuracy of ASTA MicroIDSys.

Keywords

Bacterial identification

Bruker Biotyper

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry

MicroIDSys

1. Introduction

Accurate and rapid identification of the causative organism is essential for the diagnosis and treatment of infectious diseases and is an important factor affecting patient survival and prognosis [1]. In clinical microbiology laboratories, the most commonly used methods for the identification of organisms are gram staining, morphology, and biochemical reactions. These methods are time-consuming and require a high level of expertise from the examiner. In addition, the increase in the number of elderly and immunosuppressed patients has led to a diversity of strains isolated from infectious diseases, making accurate strain identification more difficult [2-4]. Molecular diagnostic tests can be performed in cases of failure of accurate strain identification through traditional methods [5], but the testing process is time-consuming and expensive, and additional analysis of strain-specific target genes may be required in cases of small sequence differences between strains, making it unsuitable for routine use in clinical microbiology laboratories [6,7].

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is a method for analyzing the mass of constituent proteins by measuring the time it takes for an ionized sample to pass through a vacuum tube and reach a detector [8]. MALDI-TOF MS can create spectral profiles of bacterial protein expression, which can be compared to a database of bacterial protein information to identify strains. Previous studies have shown that traditional biochemical automation equipment takes about 300–480 minutes to identify strains, while MALDI-TOF MS can report strain identification results in less than 6 minutes and costs 22%–32% less [9,10]. In addition, some glucose-independent gram-negative rod bacteria, including *Streptococcus*, *Enterobacter*, and *Burkholderia cepacia* complex (BCC), have been reported to be difficult to accurately identify due to a lack of protein information or similarity in protein composition between strains [11-14], but recently it has been reported that accurate identification to the strain level is possible for most strains due to improvements in instrument resolution and databases [15]. Therefore, MALDI-TOF MS is expected to be useful in clinical microbiological laboratories due to its high concordance rate with traditional identification methods based on biochemical properties, and its simple and rapid identification of strains [16].

ASTA MicroIDSys (ASTA, Suwon, Korea) is a recently developed MALDI-TOF MS instrument for microbial species identification. It consists of a linear MALDI-TOF MS instrument with an analytical range of 2,000 to 20,000 m/z, a database containing 2,537 strains and 8,600 spectral information (as of version 1.26.02), and analysis software with an algorithm that automatically selects the number of peaks of a strain to improve accuracy, and is said to be capable of rapidly analyzing 96 to 384 samples at a time using plates of various sizes. Compared to the Bruker Biotyper (Bruker Daltonics, Bremen, Germany), which is the most widely used in clinical microbiology laboratories, high identification concordance rates have been previously reported for both aerobic bacteria and fungi and anaerobes [17-19]. In the present study, we evaluated the species identification accuracy of ASTA MicroIDSys and Bruker Biotyper on bacteria isolated in clinical microbiology laboratories during the study period. In addition, we evaluated the accuracy of species identification for genera known to have inaccurate MALDI-TOF MS results. Finally, we aimed to evaluate the utility and limitations of ASTA MicroIDSys in a typical laboratory setting.

2. Materials and methods

2.1. Target strains

All clinical isolates isolated at a university hospital in Korea from July to September 2019 were included in the study. There were 889 isolates, including 159 *Escherichia coli*, 136 *Klebsiella*, 130 *Staphylococcus*, 119 *Acinetobacter*, 84 *Enterobacter*, 69 *Enterococcus*, and 44 *Candida*, and each isolate was classified into

four groups: *Enterobacterales*, glucose non-fermenting gram-negative rods, gram-positive bacilli, and others.

2.2. MALDI-TOF MS strain identification

Species identification was performed by ASTA MicroIDSys and Bruker Biotyper using pure cultured bacterial colonies, which were tested equally by direct smear method, i.e., bacterial colonies cultured in blood agar medium for 18 hours were directly smeared on a metal plate using a wooden rod, dried, and then spotted with 1.5 μL of 70% formic acid (Sigma-Aldrich, St. Louis, MO, USA) and dried at room temperature. 1.5 μL of matrix solution (α-cyano-4-hydroxycinnamic acid saturated in 50% acetonitrile, 2.5% trifluoroacetic acid) was dropped, dried, and mounted on the instrument for examination. ASTA MicroIDSys was analyzed using database version 1.26.02 and Bruker Biotyper using Biotyper 3 software and library version 6903. Microbial identification results from MALDI-TOF MS are expressed as an identification score, which is a comparison of the mass spectrum of the sample bacterial protein with the standard mass spectrum in the database contained in the instrument. In ASTA MicroIDSys, an identification score of 140 or more indicates a high confidence in the identification result, a score of 130 or less indicates a low confidence in the identification result, and scores in between indicate the need for confirmation of the species and strain identification. Similarly, in Bruker Biotyper, an identification score of 2.30 or higher is defined as a confident strain identification result, and a score of 1.70 or lower as an unreliable identification result. In this study, strain identification results were initially reported by both instruments in order to compare analytical results under the same conditions. Therefore, the identification scores were checked against the strain results reported by the instrument, and if the ASTA MicroIDSys score was 130 or higher and the Bruker Biotyper score was 1.70 or higher, the instrument's results were reported as is, and retesting was not performed if the identification score was lower.

2.3. Identification of strains by molecular diagnostics

16S rRNA sequencing was performed on strains where ASTA MicroIDSys and Bruker Biotyper results were discordant and on clinically uncommon strains to confirm the strain. In addition, the *rpoB* gene of *Acinetobacter* and *Klebsiella* strains and the *hisA* and *recA* genes of BCC strains were additionally sequenced for accurate species identification. DNA of the strains to be sequenced was extracted using Cicageneus DNA extraction reagent ST (Kanto Chemical, Tokyo, Japan). The primers used for sequencing were as follows: 16S rRNA 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3'), the *rpoB* gene Ac696F (5'-TAYCGYAAAGAYTTGAAAGAAG-3') and Ac1093R (5'-CMACACCYTTGTTMCCRTGA-3'), the *hisA* gene A-442F (5'-AGGACCCGGCGGCGAT-3') and A-442R (5'-TGCAGCATCCCGTCGCG-3'), and the *recA* gene BCR1 (5'-TGACCGCCGAGAAGAGCAA-3') and BCR2 (5'-CTCTTCTTCGTCCATCGCCTC-3') [20-22]. Polymerase chain reaction (PCR) was performed with a C1000 Touch thermal cycler (Bio-Rad, Hercules, CA, USA), denatured the DNA at 95°C for 2 min, annealed with primers 30 cycles of 95°C for 30 s, 67°C for 45 s, and 72°C for 1 min, and amplified at 72°C for 10 min. The synthesized gene sequences were decoded using an ABI 3730xL DNA Analyzer (Applied Biosystems, Foster City, CA, USA), and the analyzed sequences were compared with the type strain database of EzBioCloud (https://www.ezbiocloud.net) and the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov) to confirm the identification results [23].

2.4. Results analysis

The reference identification, which is the basis for comparison of the target strains, was used as the reference strain name if the results of the two instruments were consistent, and the reference strain name was obtained by performing strain identification through molecular diagnostic tests if the results of the two

instruments were inconsistent. In this study, the results were classified into the following four categories for all tested strains: (1) correctly identified - species level, (2) correctly identified - genus level, (3) discrepancies down to the genus level: misidentification, and (4) failure to report an appropriate identification result (ASTA MicroIDSys strain identification score <130, Bruker Biotyper strain identification score <1.70): invalid identification. In addition, if the strain name was not in the database of the instrument, it was marked separately to compare the fidelity of the database. Finally, strain identification results reported by Bruker Biotyper in real-world clinical microbiology laboratories over the past five years were compared to the inclusion of strains in the ASTA MicroIDSys database and the percentage of agreement per strain. This allowed us to analyze the proportion of strains for which the same identification result would have been expected if the same test had been performed using ASTA MicroIDSys during this period.

3. Results

3.1. Species identification accuracy of ASTA MicroIDSys in Enterobacteriaceae strains

In a total of 418 *Enterobacterales* isolates, the genus and species identification rates were 100% (418/418) and 98.3% (411/418) for ASTA MicroIDSys and 99.5% (416/418) and 98.6% (412/418) for Bruker Biotyper, respectively (**Table 1**). ASTA MicroIDSys showed no misidentifications and invalid identifications, with two isolates of *Klebsiella variicola*, two isolates of *Enterobacter asburiae*, one isolate of *Klebsiella aerogenes*, one isolate of *Enterobacter hormaechei*, and one isolate of *Raoultella planticola*. Of these, all but one, *K. aerogenes*, had no corresponding spectrum in the database. Bruker Biotyper also had no misidentifications but showed invalid identifications for one *Klebsiella pneumoniae* and one *Proteus mirabilis*, and strain matches for one *K.variicola*, one *K. aerogenes*, one *E. hormaechei*, and one *R. planticola*.

3.2. Species identification accuracy of ASTA MicroIDSys in glucose-independent non-fermenting gram-negative bacilli

The genus and species concordance rates for ASTA MicroIDSys were 98.8% (165/167) and 91.0% (152/167), with 2 invalid identifications (1.2%) and no misidentifications (**Table 2**). Bruker Biotyper had genus and species concordance rates of 99.4% (166/167) and 88.0% (147/167), with 1 invalid identification (0.6%) and no misidentifications. ASTA MicroIDSys failed to identify one isolate of *Acinetobacter johnsonii* and one isolate of *Chryseobacterium massiliae*. For Bruker Biotyper, *Acinetobacter nosocomialis* had an invalid identification in 1 out of 28 isolates, while 5 isolates showed a genus match. In addition, one isolate of *C. massiliae* was not identified at the species level. Both instruments failed to identify three isolates of *Acinetobacter seifertii*, two isolates of *Acinetobacter soli*, one isolate of *Acinetobacter* 14TU, one isolate of *Acinetobacter grimontii*, one isolate of *Acinetobacter gyllenbergii*, one isolate of *Acinetobacter oleivorans*, one isolate of *Acinetobacter venetianus*, two isolates of *Burkholderia cepnocepacia*, and one isolate of *Burkholderia cepacian* in the species level. For *Burkholderia*, both instruments correctly identified the genus name, but only 62.5% (5/8) of the isolates were identified at the species level.

3.3. Species identification accuracy of ASTA MicroIDSys in gram-positive bacteria

For 216 isolates of gram-positive bacteria, ASTA MicroIDSys provided correct identification results down to the genus level. The Bruker Biotyper showed an invalid identification in 1 isolate of *Streptococcus pneumoniae*, resulting in a genus and species agreement rate of 99.5% (215/216) (**Table 3**).

Table 1. Comparison of bacterial identification between ASTA MicroIDSys and Bruker Biotyper for 418 *Enterobacterales* isolates

Reference identification (number of isolates)	ASTA MicroIDSys, isolates no.				Bruker Biotyper, isolates no.			
	Correctly identified		Mis-ID	Invalid-ID	Correctly identified		Mis-ID	Invalid-ID
	Genus-level	Species-level			Genus-level	Species-level		
Escherichia								
E. coli (159)	159	159			159	159		
E. hermannii (1)	1	1			1	1		
Klebsiella								
K. pneumonia (122)	122	122			121	121		1
K. oxytoca (12)	12	12			12	12		
K. varricola (2)	2	0*			2	1		
Enterobacter								
E. cloacae (45)	45	45			45	45		
E. aerogenes (36)	36	35			36	35		
E. asburiae (2)	2	0†			2	2		
E. hormaechei (1)	1	0*			1	0		
Citrobacter								
C. freundii (7)	7	7			7	7		
C. koseri (1)	1	1			1	1		
Raoultella								
R. ornithinolytica (8)	8	8			8	8		
R. planticola (1)	1	0*			1	0		
Proteus								
P. mirabilis (5)	5	5			4	4		1
P. vulgaris (1)	1	1			1	1		
Other *Enterobacterales*								
Serratia marcescens (6)	6	6			6	6		
Salmonella enterica (5)	5	5			5	5		
Providencia stuartii (2)	2	2			2	2		
Cronobacter sakazakii (1)	1	1			1	1		
Morganella morganii (1)	1	1			1	1		
Total number of isolates (%)	418 (100)	411 (98.3)			416 (99.5)	412 (98.6)		2 (0.5)

*The protein spectra of the species were not included in the database. †*E. asburiae* were reported as *E. cloacae* complex by ASTA MicroIDSys. Abbreviation: Mis-ID, misidentification; Invalid-ID, invalid identification.

Table 2. Comparison of bacterial identification between ASTA MicroIDSys and Bruker Biotyper for 167 glucose non-fermenting bacilli isolates

Reference identification (number of isolates)	ASTA MicroIDSys, isolates no.				Bruker Biotyper, isolates no.			
	Correctly identified		Mis-ID	Invalid-ID	Correctly identified		Mis-ID	Invalid-ID
	Genus-level	Species-level			Genus-level	Species-level		
Acinetobacter								
A. baumanii (47)	47	47			47	47		
A. nosocomialis (28)	28	28			27	22		1
A. pitti (13)	13	13			13	13		
A. bereziniae (8)	8	8			8	8		
A. haemolyticus (4)	4	4			4	4		
A. ursingii (4)	4	4			4	4		
A. serfertii (3)	3	0*			3	0*		
A. radioresistens (2)	2	2			2	2		
A. soli (2)	2	0*			2	0*		
A. 14TU (1) (*A. colistiniresistens*)	1	0*			1	0*		
A. baylyi (1)	1	1			1	1		
A. grimontii (1)	1	0*			1	0*		
A. gyllenbergii (1)	1	0*			1	0*		
A. johnsonii (1)	0	0		1	1	1		
A. lwoffii (1)	1	1			1	1		
A. oleivorans (1)	1	0			1	0*		
A. venetianus (1)	1	0*			1	0*		
Pseudomonas								
P. aeruginosa (23)	23	23			23	23		
Stenotrophomonas								
S. maltophilia (13)	13	13			13	13		
Burkholderia								
B. cenocepacia (4)	4	2			4	3		
B. cepacian (4)	4	3			4	2		
Other GNFB								
Chryseobacterium massiliae (1)	0	0		1*	1	0*		
Chryseobacterum gleum (1)	1	1			1	1		
Chrysobacterium indologenes (1)	1	1			1	1		
Moraxella catarrhalis (1)	1	1			1	1		
Total number of isolates (%)	165 (98.8)	152 (91.0)		2 (1.2)	166 (99.4)	147 (88.0)		1 (0.6)

*The protein spectra of the species were not included in the database. Abbreviation: Mis-ID, misidentification; Invalid-ID, invalid identification.

Table 3. Comparison of bacterial identification between ASTA MicroIDSys and Bruker Biotyper for 216 gram-positive cocci isolates

Reference identification (number of isolates)	ASTA MicroIDSys, isolates no.				Bruker Biotyper, isolates no.			
	Correctly identified		Mis-ID	Invalid-ID	Correctly identified		Mis-ID	Invalid-ID
	Genus-level	Species-level			Genus-level	Species-level		
Staphylococcus								
S. aureus (45)	45	45			45	45		
S. epidermis (42)	42	42			42	42		
S. capitis (9)	9	9			9	9		
S. haemolyticis (8)	8	8			8	8		
S. lugdunensis (8)	8	8			8	8		
S. caprae (6)	6	6			6	6		
S. hominis (5)	5	5			5	5		
S. pasteuri (3)	3	3			3	3		
S. saprophyticus (2)	2	2			2	2		
S. simulans (1)	1	1			1	1		
S. warneri (1)	1	1			1	1		
Enterococcus								
E. faecium (45)	45	45			45	45		
E. faecalis (20)	20	20			20	20		
E. avium (1)	1	1			1	1		
E. casseliflavus (1)	1	1			1	1		
E. gallinarum (1)	1	1			1	1		
E. raffinosus (1)	1	1			1	1		
Streptococcus								
S. agalactiae (5)	5	5			5	5		
S. pneumoniae (3)	3	3			2	2		1
S. anginosus (2)	2	2			2	2		
S. constellatus (1)	1	1			1	1		
S. dysgalactiae (1)	1	1			1	1		
S. gallolyticus (1)	1	1			1	1		
S. pyogenes (1)	1	1			1	1		
Other gram-positive cocci								
Lactococcus lactis (1)	1	1			1	1		
Micrococcus luteus (1)	1	1			1	1		
Total number of isolates (%)	216 (100)	216 (100)			215 (99.5)	215 (99.5)		1 (0.5)

Abbreviation: Mis-ID, misidentification; Invalid-ID, invalid identification.

3.4. Species identification accuracy of ASTA MicroIDSys in other strains

For other strains, including yeast-like fungi such as *Candida* species, gram-positive rods such as *Corynebacterium striatum*, and anaerobic bacteria such as *Bacteroides fragilis*, ASTA MicroIDSys had a 98.9% (87/88) genus and species identification rate, while Bruker Biotyper had a 98.9% (87/88) genus and 97.7% (86/88) species identification rate (**Table 4**). *Nocardia nova* resulted in an invalid identification on both instruments, and Bruker Biotyper showed a strain match for 1 isolate of *Neisseria macacae*.

Table 4. Comparison of bacterial identification between ASTA MicroIDSys and Bruker Biotyper for 88 other isolates

Reference identification (number of isolates)	ASTA MicroIDSys, isolates no.				Bruker Biotyper, isolates no.			
	Correctly identified		Mis-ID	Invalid-ID	Correctly identified		Mis-ID	Invalid-ID
	Genus-level	Species-level			Genus-level	Species-level		
Candida								
C. albicans (18)	18	18			18	18		
C. tropicalis (16)	16	16			16	16		
C. glabrata (6)	6	6			6	6		
C. krusei (2)	2	2			2	2		
C. parapsilosis (2)	2	2			2	2		
Corynebacterium								
C. striatum (11)	11	11			11	11		
C. tuberculostrearicum (2)	2	2			2	2		
C. accolens (1)	1	1			1	1		
Others								
Elizabethkingia meningoseptica (6)	6	6			6	6		
Saccharomyces cerevisiae (2)	2	2			2	2		
Actinomyces neuii (1)	1	1			1	1		
Actinomyces odontolyticus (1)	1	1			1	1		
Aeromonas caviae (1)	1	1			1	1		
Bacillus pumilus (1)	1	1			1	1		
Bacteroides fragilis (1)	1	1			1	1		
Brevibacterium casei (1)	1	1			1	1		
Campylobacter jejuni (1)	1	1			1	1		
Clostridioides difficile (1)	1	1			1	1		
Clostridium innocuum (1)	1	1			1	1		
Eggerthella lenta (1)	1	1			1	1		
Eikenella corrodens (1)	1	1			1	1		
Finegoldia magna (1)	1	1			1	1		
Haemophilus influenza (1)	1	1			1	1		
Leuconostoc lactis (1)	1	1			1	1		
Neisseria macacae (1)	1	1			1	1		
Nocardia nova (1)	0	0		1*	0	0		1
Paenibacillus amylolyticus (1)	1	1			1	1		
Pichia norvegensis (1)	1	1			1	1		
Prevotella intermedia (1)	1	1			1	1		
Propionibacterium avidum (1)	1	1			1	1		
Rothia mucilaginosa (1)	1	1			1	1		
Vibrio parahaemolyticus (1)	1	1			1	1		
Total number of isolates (%)	87 (98.9)	87 (98.9)		1 (1.1)	87 (98.9)	86 (97.7)		1 (1.1)

*Strains not in the database of the instrument. Abbreviation: Mis-ID, misidentification; Invalid-ID, invalid identification.

3.5. Strains with discrepancies between the two instruments

Out of a total of 889 isolates, 17 isolates of *Acinetobacter* species, 6 isolates of *Burkholderia* species, 4 isolates of *Klebsiella* species, 3 isolates of *Enterobacter* species, and 1 isolate each of *Chryseobacterium* species, *Neisseria* species, *Nocardia* species, *Proteus* species, *Raoultella* species, and *Streptococcus* species were identified as discrepancies (**Table 5**). There was a total of 23 discrepancies in ASTA MicroIDSys, of which 17 (73.9%) were strains not included in the database.

Table 5. List of bacterial identification differences between ASTA MicroIDSys and Bruker Biotyper

Group	Reference identification	ASTA MicroIDSys		Bruker Biotype	
		Results	ID score	Results	ID score
Enterobacterales	*Enterobacter asburiae*	***Enterobacter cloacae***†	220	*Enterobacter asburiae*	1.771
	Enterobacter asburiae	***Enterobacter cloacae***†	172	*Enterobacter asburiae*	2.109
	Enterobacter hormaechei	***Enterobacter cloacae****	146	***Enterobacter cloacae***	2.193
	Klebsiella (Enterobacter) aerogenes	***Klebsiella pneumoniae***	213	***Klebsiella variicola***	2.238
	Klebsiella pneumoniae	*Klebsiella pneumoniae*	258	**Invalid Identification**	1.663
	Klebsiella variicola	***Klebsiella pneumoniae****	223	*Klebsiella variicola*	2.138
	Klebsiella variicola	***Klebsiella pneumoniae****	211	***Klebsiella pneumoniae***	1.879
	Proteus mirabilis	*Proteus mirabilis*	236	**Invalid Identification**	1.270
	Raoultella planticola	***Raoultella ornithinolytica****	214	***Raoultella ornithinolytica***	2.331
Glucose non-fermenting bacilli	*Acinetobacter* 14TU (*A. colistiniresistens*)	***Acinetobacter junii****	188	***Acinetobacter parvus****	1.895
	Acinetobacter nosocomialis	*Acinetobacter baumannii* complex (*nosocomialis*)	155	***Acinetobacter baumannii***	2.049
	Acinetobacter nosocomialis	*Acinetobacter baumannii* complex (*nosocomialis*)	226	***Acinetobacter baumannii***	1.718
	Acinetobacter nosocomialis	*Acinetobacter baumannii* complex (*nosocomialis*)	198	***Acinetobacter baumannii***	1.985
	Acinetobacter nosocomialis	*Acinetobacter baumannii* complex (*nosocomialis*)	174	***Acinetobacter baumannii***	1.911
	Acinetobacter nosocomialis	*Acinetobacter baumannii* complex (*nosocomialis*)	164	***Acinetobacter baumannii***	2.027
	Acinetobacter nosocomialis	*Acinetobacter baumannii* complex (*nosocomialis*)	159	**Invalid Identification**	1.667
	Acinetobacter grimontii	***Acinetobacter junii****	253	***Acinetobacter junii****	2.166
	Acinetobacter gyllenbergii	***Acinetobacter junii****	181	***Acinetobacter junii****	1.851
	Acinetobacter johnsonii	**Invalid Identification**	-	*Acinetobacter johnsonii*	2.265
	Acinetobacter oleivorans	***Acinetobacter baumannii* complex (*pittii*)**	145	***Acinetobacter calcoaceticus****	1.833
	Acinetobacter seifertii	***Acinetobacter baumannii* complex (*pittii*)***	133	***Acinetobacter pittii****	1.941
	Acinetobacter seifertii	***Acinetobacter baumannii* complex (*nosocomialis*)***	161	***Acinetobacter baumannii****	1.994
	Acinetobacter seifertii	***Acinetobacter nosocomialis****	144	***Acinetobacter pittii****	1.888
	Acinetobacter soli	***Acinetobacter baylyi****	176	**Invalid Identification***	1.670
	Acinetobacter soli	***Acinetobacter baylyi****	195	***Acinetobacter baylyi****	1.821
	Acinetobacter venetianus	***Acinetobacter haemolyticus****	189	***Acinetobacter haemolyticus****	2.190
	Burkholderia cepacia	*Burkholderia cepacia*	215	***Burkholderia cenocepacia***	1.880
	Burkholderia cenocepacia	***Burkholderia cepacia***	184	*Burkholderia cenocepacia*	2.115
	Burkholderia cenocepacia	***Burkholderia ambifaria***	182	*Burkholderia cenocepacia*	2.106
	Burkholderia cepacia	***Burkholderia cenocepacia***	201	*Burkholderia cepacia*	2.232
	Burkholderia cenocepacia	*Burkholderia cenocepacia*	194	***Burkholderia cepacia***	2.169
	Burkholderia cepacia	*Burkholderia cepacia*	199	***Burkholderia seminalis***	2.102
	Chryseobacterium massiliae	**Invalid Identification***	-	***Chryseobacterium* species***	1.997
Gram-positive cocci	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*	182	**Invalid Identification**	1.670
Others	*Neisseria macacae*	*Neisseria macacae*	184	***Neisseria mucosa***	2.052
	Nocardia nova	**Invalid Identification***	-	**Invalid Identification**	1.696

The isolates which were not correctly identified at the species level were indicated in bold. *The protein spectra of the species were not included in the database. †*E. asburiae* were reported as *E. cloacae* complex by ASTA MicroIDSys. Abbreviation: ID, identification.

3.6. Comparison with mycobacteriological cultures results reported by clinical microbiology laboratories over a 5-year period

A total of 108,251 results reported by the clinical microbiology laboratory of one general hospital after species identification in bacteriological cultures over a five-year period from September 2014 to September 2019 were compared with the list of strains in the ASTA MicroIDSys database. A total of 133 genera and 401 species were reported by the laboratory, of which 120 genera (90.2%) and 349 species (87.0%) were included in the ASTA MicroIDSys database. However, the proportion of strains not included in the database in the total number of laboratory-reported identifications over the five-year period was only 0.3% (373/108,251) at the genus level and 1.4% (1,523/108,251) at the species level. Using the ASTA MicroIDSys identification concordance rates found in this study, the previously reported results were predicted to have 99.7% concordance at the strain level and 97.9% concordance at the species level (**Table 6**).

4. Discussion

Although most Enterobacteriaceae can be relatively accurately identified using automated equipment or MALDI-TOF MS using biochemical methods [24], it has been reported that MALDI-TOF MS is limited in its ability to identify strains in some species, including *Enterobacter* spp. Especially in the case of *E. cloacae*, it is known that it is not accurately distinguished by MALDI-TOF MS equipment due to the similarity in protein composition with *E. asburiae*, *E. hormaechei*, *Enterobacter kobei*, *Enterobacter ludwigii*, etc [12]. In the present study, identification discrepancies occurred in strains such as *K. aerogenes*, *K.variicola*, *E. hormaechei*, and *R. planticola* with both instruments, although these were minority strains. For Enterobacteriaceae, ASTA MicroIDSys had a concordance rate of 98.3% (411/418) and Bruker Biotyper had a concordance rate of 98.6% (412/418), and of the seven strains with discordant identifications on ASTA MicroIDSys, six were either not included in the database (4 isolates) or were lumped together and reported as *E. cloacae* complex (2 isolates). For *Enterobacter* species, *E. cloacae*, *E. kobei*, and *E. asburiae* were reported together as the *E. cloacae* complex, and *E. hormaechei* was not in the database. It is considered that the accuracy of species identification should be improved by continuously enhancing the database.

Acinetobacter spp. are among the most important nosocomial opportunistic infections, commonly causing pneumonia and sepsis in intensive care units, and are also commonly isolated from pneumonia and burn infections in patients with artificial organs. Carbapenem-resistant Acinetobacter strains are often multidrug-resistant, with simultaneous resistance to other classes of antibiotics, and are known to significantly increase mortality and hospital length of stay [25]. Especially in Korea, *A. baumannii* has a very high carbapenem resistance rate compared to *Acinetobacter* non-*baumannii* strains, so it is necessary for clinical microbiological laboratories to accurately distinguish and report *A. baumannii* among

Table 6. Estimated identification results of ASTA MicroIDSys compared to the strains reported by Bruker Biotyper over the past five years

Year	2014	2015	2016	2017	2018	2019	Total
Reported strains	18,117	19,617	18,328	18,684	20,406	13,099	108,251
Estimated number of matched reports (%)							
Genus-level	18,107 (99.9)	19,608 (100.0)	18,251 (99.6)	18,532 (99.2)	20,316 (99.6)	13,064 (99.7)	107,878 (99.7)
Species-level	17,845 (98.5)	19,309 (98.4)	18,011 (98.3)	18,272 (97.8)	19,809 (97.1)	12,773 (97.5)	106,018 (97.9)

Acinetobacter strains [26]. In the present study, the genus concordance rate of *rpoB* gene sequencing and ASTA MicroIDSys was 99.2% (118/119) and the species concordance rate was 90.8% (108/119). *A. baumannii* accounted for 39.5% (47/119) of all *Acinetobacter* isolates, all of which were correctly identified. For *Acinetobacter* non-*baumannii*, the strain concordance rate was 84.7% (61/72), with no misidentifications as *A. baumannii*. When strains not included in the database were excluded, the strain concordance rate for *Acinetobacter* non-*baumannii* increased to 96.8% (61/63). Chung *et al.* reported that by improving Bruker Biotyper's database for *Acinetobacter* strains, they were able to increase the species identification rate [27]. In this study, ASTA MicroIDSys was highly accurate in discriminating between *A. baumannii Acinetobacter* non-*baumannii* strains, suggesting that it may be sufficient to meet clinical needs.

B. cepacia is a gram-negative aerobic rod-shaped bacterium found in the environment, including soil, water, plants, and animals. It has been reported as the causative agent of cepacia syndrome (necrotizing granulomatous pneumonia and resulting bacteremia) in patients with cystic fibrosis and chronic granulomatous disease and has also been associated with nosocomial infections and nosocomial pneumonia via catheters or contaminated fluids or agents [28,29]. BCC consists of nine genetically close strains, including *B. cepacia* genomovar I and *B. cenocepacia*, *Burkholderia ambifaria*, *Burkholderia anthina*, *Burkholderia dolosa*, *Burkholderia multivorans*, *Burkholderia pyrrocinia*, *Burkholderia stabilis*, and *Burkholderia vietnamiensis*, of which *B. cenocepacia* has four subtypes with polymorphisms in the *rpoB* gene [30]. Therefore, it is known that BCC is difficult to identify to the strain level by MALDI-TOF MS or 16s rRNA and *rpoB* gene sequencing alone, and accurate strain identification is possible by gene sequencing such as *hisA* or *recA* [31]. In this study, both MALDI-TOF MS instruments correctly identified *Burkholderia* species, but the strain concordance rate was low at 62.5%, and they could not accurately distinguish between *B. cepacia* and *B. cenocepacia*. Therefore, if a strain corresponding to BCC is identified by MALDI-TOF MS, it should be reported as reliable identification results only up to the strain level, and additional sequencing of *hisA* or *recA* genes should be performed to report the correct strain for that strain.

It is known that relatively accurate identification of *Candida* and *Nocardia* strains is possible by MALDI-TOF MS [32,33]. In this study, the identification results of *Candida* strains were accurately matched between the two instruments, but *N. nova* failed to be accurately identified by both instruments. However, ASTA MicroIDSys did not have this strain in its database, and there was only one isolate of *Nocardia* strain among the collected specimens, so the possibility of coincidental errors related to specimen condition or strain preparation could not be excluded.

Of the total 401 species reported using the Bruker Biotyper in our clinical microbiology laboratory over a five-year period, 349 (87.0%) were included in the ASTA MicroIDSys database. However, assuming that the instrument was operated in a real laboratory, for approximately 100,000 identifications, ASTA MicroIDSys was expected to report 97.9% (106,018/108,251) of the same, including most major species. Given that most of the strains that were discordant with the identification results from Bruker Biotyper or molecular diagnostics were not in the database and the relatively high concordance rate for the strains that were in the database, we believe that the assay is a good representation of the major strains commonly reported in clinical microbiology laboratories.

In conclusion, the ASTA MicroIDSys, a recently introduced MALDI-TOF MS instrument, demonstrated a high identification concordance rate for most of the clinically important organisms isolated from patient specimens and can be used in clinical microbiology laboratories. Since most of the strains that failed to be identified or showed discrepancies were not in the database, it is expected that further improvements in identification performance can be achieved by expanding the database.

Disclosure statement

The authors declare no conflict of interest.

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