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Evaluation of the Bruker Biotyper Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Identification of Blood Isolates of *Acinetobacter* Species

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Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Bruker Biotyper) was able to accurately identify 98.6% (142/144) of *Acinetobacter baumannii* isolates, 72.4% (63/87) of *A. nosocomialis* isolates, and 97.6% (41/42) of *A. pittii* isolates. All *Acinetobacter junii*, *A. ursingii*, *A. johnsonii*, and *A. radioresistens* isolates ($n = 28$) could also be identified correctly by Bruker Biotyper.

Isolates of *Acinetobacter* species causing human infections predominantly belong to *Acinetobacter calcoaceticus* (genospecies 1), *A. baumannii* (genospecies 2), *A. pittii* (genospecies 3), and *A. nosocomialis* (genospecies 13TU) (1–4). Because these isolates are phenotypically similar and difficult to distinguish using traditional microbiological and biochemical tests, they are frequently reported as *A. calcoaceticus-A. baumannii* complex (ACB complex) (1–4). Although several commercially available matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) systems are rapidly emerging novel technology widely used in clinical microbiology laboratories for rapid identification of commonly encountered bacteria and fungi (5–8), few studies have compared the accuracy of different MALDI-TOF MS systems in identifying *Acinetobacter* species to the genospecies level (9–12).

A total of 286 blood isolates of ACB complex and 39 isolates of non-ACB complex that had been recovered from patients treated at the National Taiwan University Hospital from 2010 to 2012 were obtained. Conventional biochemical identification methods as well as the Phoenix bacterial identification system (NMIC/ID-72 cards; Becton, Dickinson Diagnostic Instrument Systems, Sparks, MD) and Vitek 2 (GN cards; bioMérieux, Marcy l'Etoile, France) were used for the identification of the isolates (4). We sequenced the 16S-23S rRNA gene intergenic spacer (ITS) region and a 350-bp highly variable zone of the *rpoB* gene to identify the isolates to the genospecies level (2, 4). The sequences obtained were compared with published sequences in the GenBank database using the BLASTN algorithm (http://www.ncbi.nlm.nih.gov/blast).

Bacteria were prepared for analysis by the MALDI Bruker Biotyper system as previously described (8, 10). 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 reliable identification (8, 10). Clustering analysis of

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TABLE 1 Comparison of the Bruker Biotyper MALDI TOF MS system with molecular methods in identifying 286 blood isolates of *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex

Acinetobacter species identification with identical results by molecular methods (no. of isolates)	*Acinetobacter* species identified by Bruker Biotyper system (no. of isolates)	No. (%) of isolates with the following range of scores by the Bruker Biotyper system:		
		≥2.0	1.7–1.999	<1.7
A. baumannii (144)	*A. baumannii* (142)	142 (100)	0 (0)	0 (0)
	A. nosocomialis (2)	2 (100)	0 (0)	0 (0)
A. nosocomialis (87)	*A. nosocomialis* (63)	62 (98.4)	1 (1.6)	0 (0)
	A. baumannii (22)	17 (77.3)	5 (22.7)	0 (0)
	A. pittii (2)	0 (0)	2 (100)	0 (0)
A. pittii (42)	*A. pittii* (41)	40 (97.6)	1 (2.4)	0 (0)
	A. nosocomialis (1)	1 (100)	0 (0)	0 (0)
Acinetobacter genospecies "close to 13TU" (13)[a]	*A. baumannii* (12)	10 (83.3)	2 (16.7)	0 (0)
	A. pittii (1)	1 (100)	0 (0)	0 (0)

[a] Not included in Bruker Biotyper database (DB 5627).

TABLE 2 Results of identification of 39 *Acinetobacter* species other than *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex isolates by different methods

Species no.	Species identified by gene sequencing[a]		MALDI Bruker Biotyper result[b]		Vitek 2 result		Phoenix result	
	ITS gene	*rpoB* gene	Species identified	Score	Species identified	Identity (%)[c]	Species identified	Identity (%)[c]
1	*A. junii*	*A. junii*	*A. junii*	2.351	*A. junii*	99	*Acinetobacter* sp.	90
2	*A. junii*	*A. junii*	*A. junii*	2.42	*A. junii*	99	*A. lwoffii*	90
3	*A. junii*	*A. junii*	*A. junii*	2.268	*A. junii*	99	*A. lwoffii*	99
4	*A. junii*	*A. junii*	*A. junii*	2.34	*A. junii*	98	*A. haemolyticus*	91
5	*A. junii*	*A. junii*	*A. junii*	2.462	*A. junii*	99	*A. lwoffii*	LD
6	*A. junii*	*A. junii*	*A. junii*	2.369	*A. junii*	99	*A. lwoffii*	90
7	*A. junii*	*A. junii*	*A. junii*	2.397	*A. junii*	99	*Acinetobacter* sp.	90
8	*A. junii*	*A. junii*	*A. junii*	2.346	*A. junii*	94	*Acinetobacter* sp.	92
9	*A. junii*	*A. junii*	*A. junii*	2.322	*A. junii*	98	*A. haemolyticus*	91
10	*A. junii*	*A. junii*	*A. junii*	2.315	*A. junii*	99	*A. lwoffii*	90
11	*A. junii*	*A. junii*	*A. junii*	2.323	*A. ursingii*	97	*A. lwoffii*	LD
12	*A. junii*	*A. junii*	*A. junii*	2.420	*A. junii*	98	*Acinetobacter* sp.	90
13	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.041	*A. ursingii*	96	*Acinetobacter* sp.	98
14	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.018	*A. ursingii*	97	*A. lwoffii*	90
15	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.037	*A. ursingii*	97	*Acinetobacter* sp.	90
16	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.09	*A. ursingii*	98	*Acinetobacter* sp.	90
17	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.058	*A. ursingii*	97	*A. lwoffii*	LD
18	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.116	*A. ursingii*	LD	*Acinetobacter* sp.	90
19	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.101	*A. ursingii*	98	*Acinetobacter* sp.	90
20	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.099	*A. ursingii*	97	*Acinetobacter* sp.	95
21	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.107	*A. ursingii*	97	*Acinetobacter* sp.	90
22	*A. ursingii*	*A. ursingii*	*A. ursingii*	2.098	*A. ursingii*	93	*Acinetobacter* sp.	90
23	*A. johnsonii*	*A. johnsonii*	*A. johnsonii*	2.242	*Sphingomonas paucimobilis*	86	*A. lwoffii*	90
24	*A. johnsonii*	*A. johnsonii*	*A. johnsonii*	2.373	*A. lwoffii*	97	*A. lwoffii*	90
25	*A. johnsonii*	*A. johnsonii*	*A. johnsonii*	2.390	*A. lwoffii*	LD	*A. lwoffii*	99
26	*A. radioresistens*	*A. radioresistens*	*A. radioresistens*	2.359	*A. radioresistens*	99	*A. lwoffii*	99
27	*A. radioresistens*	*A. radioresistens*	*A. radioresistens*	2.305	*A. radioresistens*	LD	*A. lwoffii*	99
28	*A. radioresistens*	*A. radioresistens*	*A. radioresistens*	2.327	*A. radioresistens*	99	*A. lwoffii*	99
29	*Acinetobacter* genospecies 14TU	*A. gyllenbergii*	*A. junii*	1.866	*A. baumannii*	99	*A. haemolyticus*	90
30	*Acinetobacter* genospecies 14TU	*Acinetobacter* genospecies 16BJ	*A. junii*	1.958	*Alcaligenes faecalis* subsp. *faecalis*	98	*A. haemolyticus*	97
31	*Acinetobacter* genospecies 14TU	*A. gyllenbergii*	*A. parvus*	1.743	*A. baumannii*	97	*Burkholderia cepacia*	98
32	*Acinetobacter* genospecies 16BJ	*Acinetobacter* genospecies 16BJ	*A. junii*	1.857	*A. junii*	99	*A. haemolyticus*	LD
33	*Acinetobacter* genospecies 16BJ	*Acinetobacter* genospecies 16BJ	*A. haemolyticus*	1.858	*Pseudomonas pseudoalcaligenes*	97	*A. haemolyticus*	95
34	*Acinetobacter* genospecies 16BJ	*Acinetobacter* genospecies 16BJ	*A. junii*	2.035	*A. ursingii*	LD	*A. haemolyticus*	98
35	*A. tjernbergiae*	*Acinetobacter* genospecies 14TU	*A. junii*	1.914	*A. baumannii*	99	*A. haemolyticus*	90
36	*A. tjernbergiae*	*A. parvus*	*A. junii*	1.921	*A. ursingii*	98	*A. lwoffii*	90
37	*A. bereziniae*	*A. bereziniae*	*Acinetobacter* sp.	1.741	*A. lwoffii*	99	ACB complex	99
38	*A. guillouiae*	*A. radioresistens*	*A. guillouiae*	1.914	*A.baumannii*	99	ACB complex	92
39	*A. haemolyticus*	*A. junii*	*A. junii*	2.279	*A. junii*	99	*A. lwoffii*	90

[a] Species are identified by maximal identification of gene sequencing.
[b] Bruker Biotyper database (DB 5627) does not include *Acinetobacter* genospecies 14TU, *Acinetobacter* genospecies 16BJ, *A. bereziniae* (*Acinetobacter* genospecies 10), and *A. gyllenbergi*.
[c] LD, low discrimination.

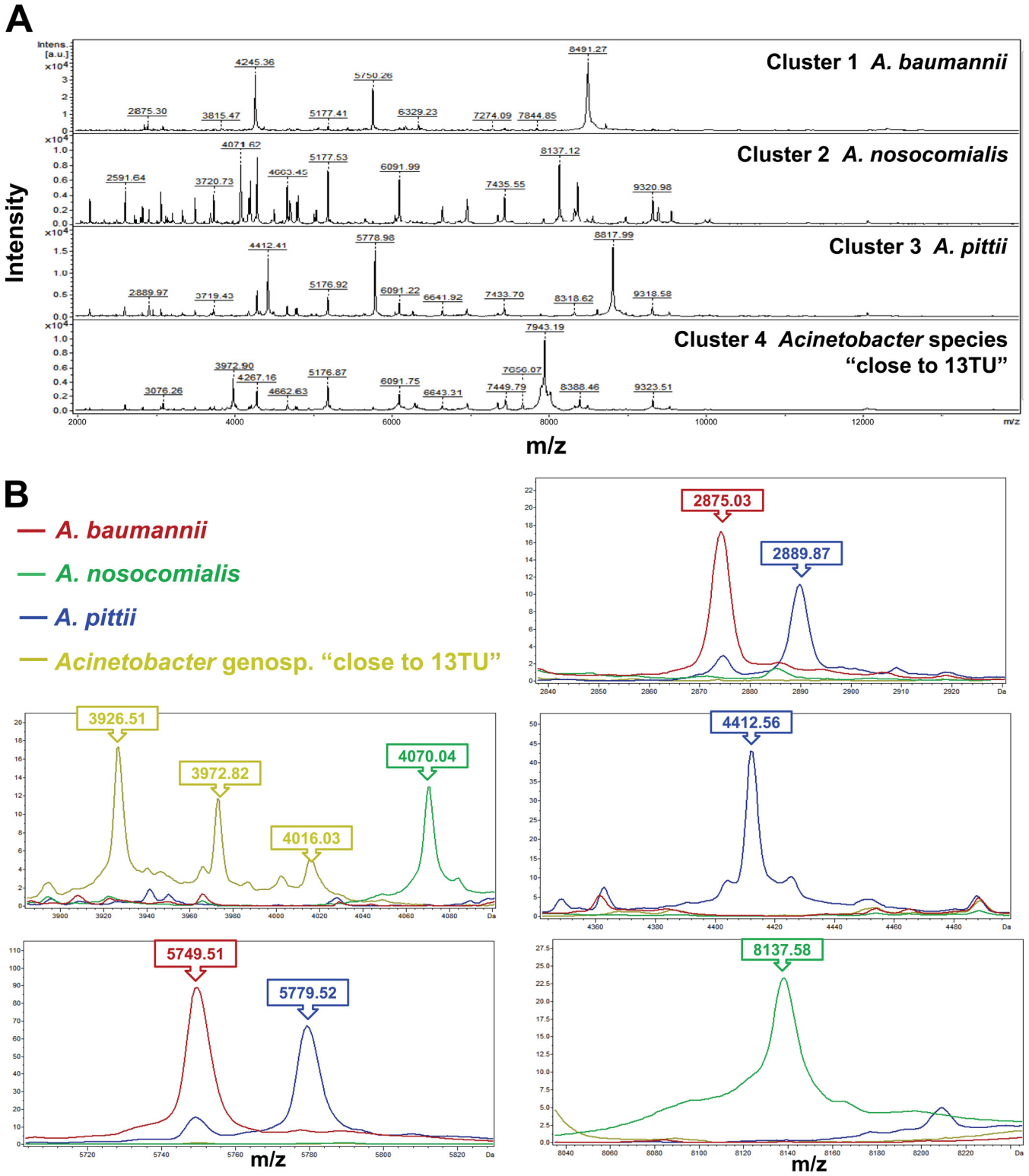

FIG 1 Cluster analysis of spectra generated by the MALDI-TOF Bruker Biotyper system for 286 *A. baumannii* complex (ACB complex) isolates. (A) Four clusters of spectra for ACB complex isolates, i.e., cluster 1 (*A. baumannii*), cluster 2 (*A. nosocomialis*), cluster 3 (*A. pittii*), and cluster 4 (*Acinetobacter* genospecies "close to 13TU") were identified. (B) Clusters were based on the 10 peaks generated by ClinProTools with a genetic algorithm, i.e., 2875.03 *m/z* and 5749.51 *m/z* (*A. baumannii*)), 4070.04 *m/z* and 8137.58 *m/z* (*A. nosocomialis*); 2889.87 *m/z*, 4412.56 *m/z*, and 5779.52 *m/z* (*A. pittii*); and 3926.51 *m/z*, 3972.82 *m/z*, and 4016.03 *m/z* (*Acinetobacter* genospecies "close to 13TU"). 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* values represent the mass-to-charge ratios.

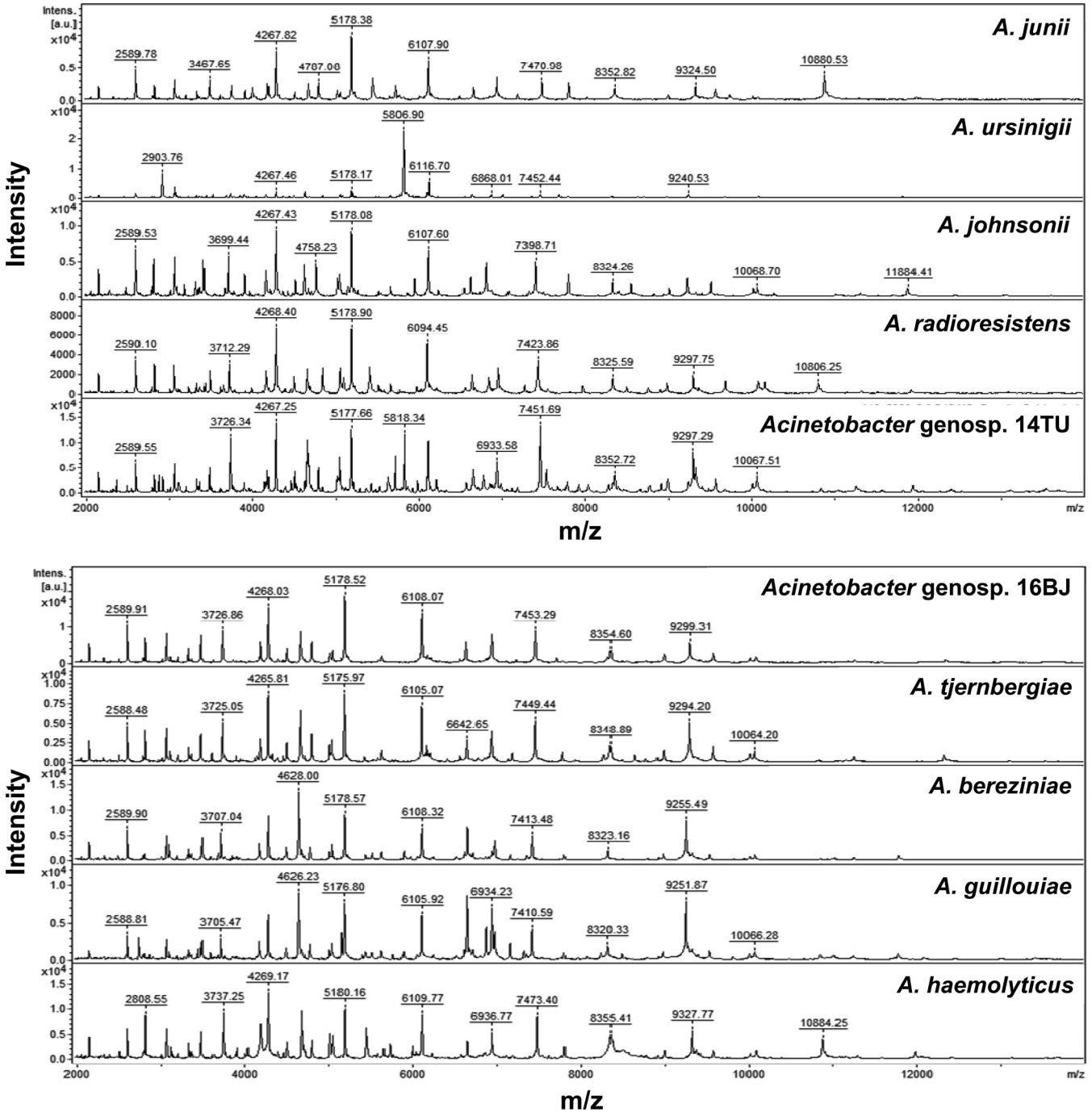

FIG 2 Spectra of 10 species (genospecies) of *Acinetobacter* species other than ACB complex generated by the MALDI-TOF Bruker Biotyper system. The absolute intensities of the ions are shown on the *y* axis, and the masses (*m/z*) of the ions are shown on the *x* axis. The *m/z* values represent the mass-to-charge ratios.

four genetically identified genospecies of ACB complex was performed using ClincProtools 3.0 (Bruker Daltonics GmbH, Bremen, Germany) (8). Characteristic peaks of the spectra of the four genospecies of ACB complex were identified. Cluster analysis of half of the *A. baumannii* isolates identified by the Bruker Biotyper system was also performed (8). The remaining half of the *A. baumannii* isolates identified by the Bruker Biotyper system were used as external validation isolates.

Tables 1 and 2 show the results of two molecular methods for identification of the 286 isolates of ACB complex and 39 non-ACB complex isolates. No discrepant results were found for ACB complex isolates by the two molecular identification methods (Table 1). The Phoenix and Vitek 2 systems identified all isolates of ACB complex as *A. baumannii* (complex). The overall accuracy rate of identifying *Acinetobacter* species other than ACB complex was higher in the Vitek 2 system (61.5% [24/39]) than in the Phoenix system (0%). Vitek 2 had good identification results for *A. junii* (91.7%), *A. ursingii* (100%), and *A. radioresistens* (100%). Of the 14 species (genospecies) of *Acinetobacter* isolates evaluated in the present study, *Acinetobacter* genospecies "close to 13TU," *Acineto-*

TABLE 3 Results of external validation of 87 spectra of the three genospecies of the *A. baumannii* complex (ACB complex) isolates by the Bruker Biotyper system

Acinetobacter species	No. of isolates	No. (%) of isolates with the following genospecies of ACB complex by external validation[a]:		
		A. baumannii	*A. nosocomialis*	*Acinetobacter* genospecies 13TU
A. baumannii	70	**69 (98.6)**	1 (1.4)	0
A. nosocomialis	11	0	**11 (100)**	0
Acinetobacter genospecies "close to 13TU"	6	1 (3.3)		**6 (100)**

[a] The rates of correct identification by external validation results are given in boldface type.

bacter genospecies 14TU, *Acinetobacter* genospecies 16BJ, *A. bereziniae* (*Acinetobacter* genospecies 10), and *A. gyllenbergii* are not included in the Bruker Biotyper database (DB 5627). Of the 286 isolates of ACB complex, the accuracy rate of genospecies identification by the Bruker Biotyper system was 98.6% (142/144) for *A. baumannii*, 72.4% (63/87) for *A. nosocomialis*, and 97.6% (41/42) for *A. pittii*. Twelve of the 13 isolates of *Acinetobacter* genospecies "close to 13TU" were identified as *A. baumannii*, and 10 of these isolates had score values of ≥2.000 (Table 1).

Of the 286 ACB complex isolates, 176 were identified as *A. baumannii* by the Bruker Biotyper system, and 142 (80.7%) were genetically confirmed to be *A. baumannii*, 22 (12.5%) were confirmed to be *A. nosocomialis*, and 12 (6.8%) were confirmed to be *Acinetobacter* genospecies "close to 13TU." Of the 66 isolates of *A. nosocomialis* identified by the Bruker Biotyper, 63 (95.5%) were identified correctly. Of the 44 isolates of *A. pittii* identified by the Bruker Biotyper, three (6.8%) were not correctly identified by molecular methods as being *A. pittii*. Of the 39 isolates of *Acinetobacter* species other than ACB complex, accurate identification by the Bruker Biotyper was obtained for all *A. junii*, *A. ursingii*, *A. johnsonii*, and *A. radioresistens* isolates (Table 2).

Cluster analysis of spectra generated by the Bruker Biotyper for 286 isolates of four genospecies of ACB complex isolates was performed. Four clusters of spectra for ACB complex isolates, i.e., cluster 1 (*A. baumannii*), cluster 2 (*A. nosocomialis*), cluster 3 (*A. pittii*), and cluster 4 (*Acinetobacter* genospecies "close to 13TU") were identified based on the 10 peaks generated by ClinProTools with the genetic algorithm, i.e., 2875.03 *m/z* and 5749.51 *m/z* (*A. baumannii*), 4070.04 *m/z* and 8137.58 *m/z* (*A. nosocomialis*); 2889.87 *m/z*, 4412.56 *m/z* and 5779.52 *m/z* (*A. pittii*); and 3926.51 *m/z*, 3972.82 *m/z*, and 4016.03 *m/z* (*Acinetobacter* genospecies "close to 13TU") (Fig. 1A and B). The spectra of 10 species (genospecies) of *Acinetobacter* species other than ACB complex generated by the MALDI-TOF Bruker Biotyper system are shown in Fig. 2.

Of the 286 ACB complex isolates, 176 were identified as *A. baumannii* by Bruker Biotyper. Of these 176 isolates, 142 (80.7%) were genetically confirmed to be *A. baumannii*, 22 (12.5%) were confirmed to be *A. nosocomialis*, and 12 (6.8%) were confirmed to be *Acinetobacter* genospecies "close to 13TU". Cluster analysis of spectra generated by the Bruker Biotyper system for model establishment was performed for nearly half ($n = 89$) of the 176 isolates of *A. baumannii* identified by the Bruker Biotyper system. Genetically, 72 were *A. baumannii*, 11 were *A. nosocomialis*, and 6 were *Acinetobacter* genospecies "close to 13TU". The characteristic signals generated using clustering analysis by ClinProTools with the genetic algorithm specific for separating the three genospecies of ACB complex were 3091.35 *m/z*, 3926.18 *m/z*, 4070.04 *m/z*, 5749.51 *m/z*, and 8137.58 *m/z*. Of the remaining 87 BioTyper spectra of *A. baumannii* isolates used for external validation, 70 were genotypically confirmed *A. baumannii* isolates, 11 were *A. nosocomialis* isolates, and 6 were *Acinetobacter* genospecies "close to 13TU." The overall correct validation rates by the established model were 98.8% for the three genospecies of the ACB complex (Table 3).

Several important findings were demonstrated. First, the overall rate of the Bruker Biotyper in correctly identifying ACB complex isolates to the species level (score values ≥2.0) was only 85.3%. This is partly because *Acinetobacter* species 13TU is not included in the current database. Using cluster analysis of spectra from the 286 isolates of the ACB complex, 10 peaks were identified to be unique to the four genospecies and might be useful to generate a local database. Second, although *A. nosocomialis* is included in the current database of the Bruker Biotyper system, the correct identification rate was only 72.4%. Third, of the 176 *A. baumannii* isolates identified by the Bruker Biotyper system (score values of ≥1.700), about 80% were correctly identified to the species level (score values of ≥2.000). Interestingly, using six characteristic markers created by cluster analysis of spectra of the 176 isolates, nearly all (98.8%) could be reidentified correctly. Finally, compared with the Vitek identification system, the Phoenix identification system is not suitable for identification of *Acinetobacter* species, particularly for species other than ACB complex isolates.

Alvarez-Buylla et al. reported that the MALDI-TOF Bruker Biotyper system is useful for identifying *A. baumannii* and *A. pittii* but not for identifying *A. calcoaceticus*, *A. nosocomialis*, *A. berezinae*, or *A.* genospecies 14BJ (10). The total misidentification rate by the MALDI-TOF Bruker Biotyper system was 16.4% for all *Acinetobacter* species tested compared with the identification results by *rpoB* gene sequencing analysis. Espinal et al. analyzed 60 genotypically characterized ACB complex isolates, including 18 *A. nosocomialis* isolates, 17 *A. pittii* strains, 18 *A. baumanni* isolates, and 7 other *Acinetobacter* species isolates against a local database (inclusion of specific signature profiles for *A. nosocomialis* within the Bruker database) and found that 98.3% of all isolates were identified at the species level (log score > 2.0), and only 1.7% (one of seven other *Acinetobacter* species) were identified at the probable genus level with a log score between 1.7 and 2.0 (9).

Our work documented high identification rates of *A. baumannii*, *A. pittii*, and non-ACB complex by the MALDI-TOF Bruker Biotyper system. However, further expansion and improvement of the database, including *A. nosocomialis* and *Acinetobacter* genospecies "close to 13TU," is mandatory to make MALDI-TOF MS an efficient method for identification of all *Acinetobacter* species.

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