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Evaluation of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based VITEK MS System for the Identification of *Acinetobacter* Species from Blood Cultures: Comparison with VITEK 2 and MicroScan Systems

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Background: *Acinetobacter* species are the leading cause of bloodstream infection (BSI), but their correct identification is challenging. We evaluated the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)-based VITEK MS (bioMérieux, France), and two automated systems, VITEK 2 (bioMérieux) and MicroScan (Siemens, USA) for identification of *Acinetobacter* BSI isolates.

Methods: A total of 187 BSI isolates recovered at a university hospital in Korea between 2010 and 2012 were analyzed. The identification results obtained using VITEK MS and two automated systems were compared with those of *rpoB* sequencing.

Results: Of 187 isolates analyzed, 176 were identified to the species level by *rpoB* sequencing: the *Acinetobacter baumannii* group (ABG; 101 *A. baumannii*, 43 *A. nosocomialis*, 10 *A. pittii* isolates) was most commonly identified (82.4%), followed by *Acinetobacter* genomic species 13BJ/14TU (5.3%), *A. ursingii* (2.1%), *A. soli* (2.1%), *A. bereziniae* (1.1%), and *A. junii* (1.1%). Correct identification rates to the species group (ABG) level or the species level was comparable among the three systems (VITEK MS, 90.3%; VITEK 2, 89.2%; MicroScan, 86.9%). However, VITEK MS generated fewer misidentifications (0.6%) than VITEK 2 (10.8%) and MicroScan (13.1%) ($P<0.001$). In addition, VITEK MS demonstrated higher specificity (100%) for discrimination between ABG and non-ABG isolates than the other systems (both, 31.8%) ($P<0.001$).

Conclusions: The VITEK MS system is superior to the VITEK 2 and MicroScan systems for identification of *Acinetobacter* BSI isolates, with fewer misidentifications and better discrimination between the ABG and non-ABG isolates.

Key Words: VITEK MS, VITEK 2, MicroScan, *Acinetobacter*, Identification

INTRODUCTION

Acinetobacter species are one of the leading causative agents of healthcare-associated infections worldwide, including bloodstream infection (BSI), pneumonia, urinary tract infection, and meningitis [1-3]. To date, more than 32 species of the *Acinetobacter* genus have been identified by gene sequencing [4-8]. Members of the *Acinetobacter baumannii* group (ABG), which

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consists of *A. baumannii*, *A. pittii*, and *A. nosocomialis*, share important clinical and epidemiological characteristics that cannot be distinguished by most of the currently available phenotypic identification (ID) systems [2]. Although the ABG remains the most common *Acinetobacter* species recovered from clinical specimens, the non-ABG species are also often clinically relevant [3, 4, 9-11]. Because *Acinetobacter* species may differ in their pathogenicity, epidemiology, antimicrobial susceptibility, and clinical outcomes [5, 9, 11-13], information on the relative frequency of various *Acinetobacter* species causing BSI may be useful for establishing protocols for infection control and treatment of *Acinetobacter* BSI. However, to date, data regarding distributions of BSI isolates of *Acinetobacter* species in hospitals are limited, because the correct ID of *Acinetobacter* isolates is difficult to determine when using the currently available, common phenotypic methods [2-4, 9, 10, 14].

Recent studies have shown that matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) offers an opportunity for rapid, cost-effective, convenient, and high-throughput bacterial ID in routine diagnostic procedures conducted in clinical microbiology laboratories [15, 16]. However, information regarding the suitability of the MALDI-TOF MS-based VITEK MS system (VITEK MS; bioMérieux, Marcy l'Etoile, France) for the ID of clinical isolates of *Acinetobacter* species is scarce as compared to that of other commonly used automated ID systems. In this study, we performed molecular ID to investigate the rank order of occurrence of the various species of *Acinetobacter* isolated from blood cultures obtained from a university hospital in Korea between 2010 and 2012. We performed a comparative evaluation of MALDI-TOF MS-based VITEK MS system versus the VITEK 2 (VITEK2 XL; bioMérieux) and MicroScan (MicroScan WalkAway-96 Plus; Siemens, Deerfield, IL, USA) automated systems for the correct ID of *Acinetobacter* BSI isolates, including evaluation of their discriminative abilities for the ABG versus other (non-ABG) *Acinetobacter* species.

METHODS

1. *Acinetobacter* isolation and molecular ID

A total of 187 isolates molecularly identified as belonging to the genus *Acinetobacter* were analyzed in this study. All isolates were obtained from the blood cultures of 187 patients at the Chonnam National University Hospital (a 1,000-bed tertiary-care hospital in Gwangju, Korea) between January 2010 and December 2012. Duplicate isolates of *Acinetobacter* species from the same patient were excluded. For molecular ID, DNA

was extracted from the isolates as described previously [17], and a 450-bp sequence (zone 2) of the *rpoB* gene region of each isolate was sequenced [18]. The primers Ac1055F (5´-GT-GATAARATGGCBGGTCGT-3´) and Ac1598R (5´-CGBGCRTG-CATYTTGTCRT-3´) were used to amplify the *rpoB* region. All loci were sequenced in both the forward and reverse directions with the same primers as those used for amplification. The amplification products were purified and sequenced by using an ABI 3730XL sequencer (Applied Biosystems, Foster City, CA, USA). Sequence data were assembled and compared with previously reported sequences by using the basic local alignment search tool (BLAST) of the national center for biotechnology information (NCBI) database (http://www.ncbi.nlm.nih.gov/blast).

2. ID using the VITEK MS, VITEK 2, and MicroScan systems

ID with VITEK MS (*in vitro*-diagnostic [IVD] mode) was performed according to the manufacturer's instructions by directly smearing an overnight-cultured bacterial specimen onto disposable target slides with a 1.0-μL matrix solution (VITEK MS-CHCA). *Escherichia coli* ATCC 8739 was used as the calibration strain. The advanced spectrum classifier software of the VITEK MS system proposes 3 confidence levels: (a) single choice, with one significant choice (confidence value ≥ 60); (b) low discrimination, with more than one significant choice (maximum 4 choices); and (c) unidentified organism, with no significant choice (no class with probability and score higher than the defined thresholds) or a number of significant choices greater than the defined threshold of low discrimination.

ID using the VITEK 2 and MicroScan systems was performed by using GN ID Card (bioMérieux) and Gram Negative Breakpoint Combo Panel Type 42 (Siemens), respectively, according to the manufacturers' instructions. The ID results from these systems are proposed automatically by the respective accompanying software. The tests were repeated only if the initial results indicated "low discrimination" or "no ID", and the repeat result was used for data analysis.

3. Data analysis

The ID capability of the 3 systems was evaluated by using the *Acinetobacter* isolates that were identified to the species level by *rpoB* sequencing. The ID results from the 3 systems were classified into 3 categories by comparison with the results of the reference *rpoB* sequencing: (a) "correct ID" to the species (identical to sequence-based ID) or species group level; (b) "mis-ID", when the ID result of the system differed from that of the sequence-based reference method; and (c) "no ID", when the

system could not identify the isolate. Because the ID result of the isolates belonging to the ABG (isolates of *A. baumannii*, *A. pittii*, or *A. nosocomialis*) is assigned by these 3 systems at the species group level only, the ABG isolates that were identified as "*A. baumannii* complex" by using VITEK MS IVD and VITEK 2, or those identified as "*A. baumannii/haemolyticus*" by using the MicroScan system were categorized under the "correct ID" category. Statistical analysis to compare the ID performance of the 3 systems was performed with the chi-square test, Fisher's exact test, and McNemar test using the IBM SPSS Statistics (version 21, IBM, Armonk, NY, USA) and GraphPad Prism (version 5, GraphPad Software, San Diego, CA,USA) software packages.

RESULTS

1. *Acinetobacter* isolation and molecular ID
Table 1 presents the species distribution of the 187 *Acinetobacter* BSI isolates determined by partial *rpoB* sequencing. Of the 187 isolates collected during the 3-yr period, 154 (82.4%) isolates belonged to the ABG, which included 101 (54.0% of the total 187 *Acinetobacter* isolates) *A. baumannii*, 43 (23.0%) *A. nosocomialis*, and 10 (5.3%) *A. pittii* isolates. The remaining 33 (17.6%) isolates belonged to the non-ABG: 10 (5.3%) isolates of *Acinetobacter* genomic species 13BJ/14TU, 4 (2.1%) of *A. ursingii*, 4 (2.1%) of *A. soli*, 2 (1.1%) of *A. bereziniae*, 2

(1.1%) of *A. junii*, and 11 (5.9%) of other miscellaneous *Acinetobacter* species, which were identified as the genus *Acinetobacter* but not identified to the species level. Overall, a total of 176 isolates were identified to the species level by partial *rpoB* sequencing.

2. ID using the VITEK MS, VITEK 2, and MicroScan systems
Details of the ID results from *rpoB* sequencing and the VITEK MS, VITEK 2, and MicroScan systems are provided in Table 2. Of the 154 ABG isolates, 153 (99.4%) were correctly identified by all 3 systems. The VITEK MS system correctly identified all 4 *A. ursingii* isolates and 2 *A. junii* isolates, and VITEK 2 correctly identified 2 of 4 *A. ursingii* isolates and 2 *A. junii* isolates, which were present in their respective databases; by contrast, the MicroScan system misidentified each of the 6 isolates absent from its database. Among the 16 isolates of *Acinetobacter* genomic species 13BJ/14TU, *A. soli*, and *A. bereziniae*, which were absent in the databases of the 3 systems, VITEK MS showed 1 (6.3%) mis-ID and 15 (93.8%) no ID results, whereas both VITEK 2 and MicroScan showed 16 (100%) mis-ID and 0 (0%) no ID results (*P* < 0.001).

3. ID results for the ABG and non–ABG isolates
The ID results from the 3 tested systems for the 154 ABG and 22 non-ABG isolates are summarized in Table 3. All 3 systems

Table 1. Species distribution of 187 *Acinetobacter* bloodstream isolates recovered during the 3-yr period

Final identification*	N (%) of isolates identified in each year			Total N (%) of isolates
	2010	2011	2012	
A. baumannii group				
A. baumannii	18	37	46	101 (54.0)
A. nosocomialis	18	15	10	43 (23.0)
A. pittii	4	3	3	10 (5.3)
Total	40 (88.9)	55 (83.3)	59 (77.6)	154 (82.4)
Non-*A. baumannii* group				
Acinetobacter genomic species 13BJ/14TU	2	2	6	10 (5.3)
A. ursingii	0	2	2	4 (2.1)
A. soli	0	3	1	4 (2.1)
A. bereziniae	0	1	1	2 (1.1)
A. junii	0	2	0	2 (1.1)
Other *Acinetobacter* species†	3	1	7	11 (5.9)
Total	5 (11.1)	11 (16.7)	17 (22.4)	33 (17.6)
All isolates, total	45 (100)	66 (100)	76 (100)	187 (100)

*The final identification results were obtained by *rpoB* sequencing; †Isolates were identified as belonging to genus *Acinetobacter*, but not identified up to the species level by *rpoB* sequencing.

Table 2. Detailed identification results by *rpoB* sequencing and the MALDI-TOF MS-based VITEK MS, VITEK 2, and MicroScan systems for 176 *Acinetobacter* blood isolates

Sequence-based identification (Total N of isolates)	N of isolates	VITEK MS	VITEK 2	MicroScan
A. baumannii (101)	100	*A. baumannii* complex	*A. baumannii* complex	*A. baumannii/haemolyticus*
	1	*A. baumannii* complex	*A. baumannii* complex	*Pseudomonas oryzihabitans*
A. nosocomialis (43)	43	*A. baumannii* complex	*A. baumannii* complex	*A. baumannii/haemolyticus*
A. pittii (10)	8	*A. baumannii* complex	*A. baumannii* complex	*A. baumannii/haemolyticus*
	1	No identification	*A. baumannii* complex	*A. baumannii/haemolyticus*
	1	*A. baumannii* complex	*Alcaligenes faecalis* subsp. *faecalis*	*A. baumannii/haemolyticus*
Acinetobacter genomic species 13BJ/14TU*[†] (10)	7	No identification	*A. baumannii* complex	*A. baumannii/haemolyticus*
	2	No identification	*A. baumannii* complex	*Providencia stuartii*
	1	No identification	*A. lwoffii*	*A. baumannii/haemolyticus*
A. ursingii[†] (4)	2	*A. ursingii*	*A. ursingii*	*A. lwoffii*
	1	*A. ursingii*	*A. baumannii* complex	*A. baumannii/haemolyticus*
	1	*A. ursingii*	*A. baumannii* complex	*A. lwoffii*
A. soli[*†] (4)	3	No identification	*A. baumannii* complex	*A. baumannii/haemolyticus*
	1	*A. radioresistens*	*A. baumannii* complex	*A. baumannii/haemolyticus*
A. bereziniae[*,†] (2)	1	No identification	*A. lwoffii*	*A. baumannii/haemolyticus*
	1	No identification	*A. lwoffii*	*A. lwoffii*
A. junii[†] (2)	1	*A. junii*	*A. junii*	*A. baumannii/haemolyticus*

*Species not included in the databases of VITEK MS and VITEK 2 systems; [†]Species not included in the database of MicroScan system.
Abbreviation: MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry.

Table 3. Identification results of 176 *Acinetobacter* bloodstream isolates by the MALDI-TOF MS-based VITEK MS, VITEK 2, and MicroScan systems as compared to sequence-based identification

Final identification	N of isolates	N (%) of isolates with the indicated ID result*								
		VITEK MS			VITEK 2			MicroScan		
		Correct ID	Mis-ID	No ID	Correct ID	Mis-ID	No ID	Correct ID	Mis-ID	No ID
A. baumannii group[†]	154	153 (99.4)	0 (0)	1 (0.6)	153 (99.4)	1 (0.6)	0 (0)	153 (99.4)	1 (0.6)	0 (0)
Acinetobacter genomic species 13BJ/14TU[‡§]	10	0	0	10	0	10	0	0	10	0
A. ursingii[§]	4	4	0	0	2	2	0	0	4	0
A. soli[‡§]	4	0	1	3	0	4	0	0	4	0
A. bereziniae[‡§]	2	0	0	2	0	2	0	0	2	0
A. junii[§]	2	2	0	0	2	0	0	0	2	0
Non-*A. baumannii* group, subtotal	22	6 (27.3)	1 (4.5)	15 (68.2)	4 (18.2)	18 (81.8)	0 (0)	0 (0)	22 (100)	0 (0)
All isolates, total	176	159 (90.3)	1 (0.6)	16 (9.1)	157 (89.2)	19 (10.8)	0 (0)	153 (86.9)	23 (13.1)	0 (0)

*Correct ID (identification) included "correct ID" up to the (i) species level; (ii) species group level for the *A. baumannii* group (e.g., "*A. baumannii* complex" by using VITEK MS and VITEK 2 or "*A. baumannii/haemolyticus*" by using MicroScan); [†]*A. baumannii*, *A. pittii*, and *A. nosocomialis* were included in the *A. baumannii* group; [‡]Species not included in the databases of VITEK MS and VITEK 2 systems; [§]Species not included in the database of the MicroScan system.
Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption ionization–time of flight mass spectrometry; correct ID, identification up to the species or species group level; mis-ID, identification result of the system differed from that of the sequence-based reference method; no ID, the system could not identify the isolate.

correctly identified 99.4% (153/154) of the ABG isolates. However, the test results for the 22 non-ABG isolates, including 10 *Acinetobacter* genomic species 13BJ/14TU, 4 *A. ursingii*, 4 *A.* *soli*, 2 *A. bereziniae*, and 2 *A. junii* isolates, showed correct ID rates of 27.3%, 18.2%, and 0% for the VITEK MS, VITEK 2, and MicroScan systems, respectively (VITEK MS and VITEK 2 versus

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MicroScan, $P<0.05$). The mis-ID rate for the 22 non-ABG isolates was 4.5% (1/22) using VITEK MS, which was significantly lower than that obtained using VITEK 2 (81.8%) and MicroScan (100%) ($P<0.001$). Of the 22 non-ABG isolates, no (0.0%) and 15 (68.2%) isolates were misidentified as *A. baumannii* complex when using VITEK MS and VITEK 2, respectively, and 15 (68.2%) isolates were misidentified as *A. baumannii/haemolyticus* with MicroScan. Therefore, the specificity of VITEK MS for discrimination between the ABG and non-ABG isolates was much higher (100% [22/22]) than that of VITEK 2 and MicroScan (both, 31.8% [7/22]; $P<0.001$), while the sensitivity of the 3 systems was identical at 99.4% (153/154). The false-positive rate of VITEK MS for discrimination between the ABG and non-ABG isolates was 0% (0/22), while that of both VITEK 2 and MicroScan was 68.2% (15/22) ($P<0.001$).

Overall, for all 176 isolates tested, the correct ID rates obtained with the VITEK MS, VITEK 2, and MicroScan systems were 90.3%, 89.2%, and 86.9%, respectively, which indicate similar rates of correct ID among the 3 systems. However, VITEK MS showed a lower mis-ID rate (0.6%) than VITEK 2 (10.8%) and MicroScan (13.1%) ($P<0.001$) and a higher no ID rate (9.1%) than VITEK 2 and MicroScan (both, 0%) ($P<0.001$).

DISCUSSION

To the best of our knowledge, this study reports the first comparative evaluation of MALDI-TOF MS-based VITEK MS with both the VITEK 2 and MicroScan systems for the ID of *Acinetobacter* species from BSI isolates. The current findings demonstrate that the mis-ID rate of VITEK MS was significantly lower (0.6%) than that obtained with the VITEK 2 (10.8%) and MicroScan (13.1%) systems for the ID of *Acinetobacter* BSI isolates. Moreover, VITEK MS showed the best discrimination between the ABG and non-ABG isolates in this study. Thus, we believe that VITEK MS is a useful system for the rapid and accurate ID of BSI isolates of *Acinetobacter* species in routine diagnostic procedures conducted in clinical microbiology laboratories.

For molecular ID, we analyzed a partial sequence of the *rpoB* gene region, which has a higher discriminatory power for routine ID of different *Acinetobacter* species compared to the 16S *rRNA* gene [18, 19]. Sequence-based analysis of 187 BSI isolates recovered during the 3-yr period (2010–2012) revealed that the ABG isolates represented 82.4% of all *Acinetobacter* BSI cases in our hospital. This finding, together with data from other countries, confirmed that the ABG continues to be the leading cause of *Acinetobacter* BSI worldwide [4, 9, 10]. Although *A. bauman-*

nii is the most frequent etiological agent of *Acinetobacter* BSI in our hospital, the prevalence of *A. baumannii* (54.0%) detected in this study was slightly lower than that reported for the USA (63.4%) [9], but higher than that reported for Norway (8.8%) and Japan (17.9%) in previous studies [4, 10]. Karah *et al.* [4] reported that the most prevalent species was *A. nosocomialis* (46.9%), followed by *A. pittii* (19.5%) and *A. baumannii* (8.8%) among the *Acinetobacter* BSI isolates in Norway. On the other hand, Kishii *et al.* [10] reported that the most prevalent species was *A. pittii* (34.1%), followed by *A. baumannii* (17.9%) and *A. nosocomialis* (15.4%) in Japan. Our recent report revealed that *Acinetobacter* genomic species 13BJ/14TU is innately resistant to colistin, but susceptible to most of the other clinically relevant antimicrobial agents, and that BSI patients infected with this species show excellent clinical outcomes with cleared bacteremia [11]. In the current study, among the 33 (17.6%) non-ABG isolates, 10 (5.3%) isolates of *Acinetobacter* genomic species 13BJ/14TU were identified as causative agents of BSI. Overall, our results, together with data from other countries reported previously, demonstrate that the rank order of the occurrence of various *Acinetobacter* species that cause BSI may differ among hospitals and countries and with respect to time [4, 9, 10].

To date, few evaluations of the VITEK MS system using molecularly identified *Acinetobacter* clinical isolates have been performed, with the exception of one multi-center study for the ID of non-*Enterobacteriaceae* gram-negative bacilli isolated from various samples [16]. In this previous study, when the VITEK MS ID results of 108 *Acinetobacter* isolates were compared with those determined by gene sequencing, the rates of correct ID to the species level, mis-ID, and no ID were 83.3% (90/108), 0.9% (1/108), and 13.0% (14/108), respectively. Similarly, we found that the rates of correct ID, mis-ID, and no ID using VITEK MS for 176 *Acinetobacter* BSI isolates were 90.3%, 0.6%, and 9.1%, respectively. In addition, the current study demonstrated that the VITEK MS system was better at correctly identifying the ABG isolates (99.4%) than the non-ABG isolates (27.3%). In our study, all isolates of *A. ursingii* and *A. junii*, which are included in the VITEK MS database, were correctly identified. Because the isolates described herein represent almost all of the *Acinetobacter* BSI isolates encountered in a single hospital over a 3-yr period, our finding that VITEK MS showed a lower correct ID rate for the non-ABG isolates than for the ABG isolates will provide valuable information for the diagnosis and treatment of BSI patients infected with *Acinetobacter* species.

To date, no comparative evaluation of the VITEK MS, VITEK 2, and MicroScan systems for the ID of BSI isolates of *Acineto-*

bacter species has been reported. The present study revealed that the correct ID rate of VITEK MS (90.3%) was comparable to that of the VITEK 2 (89.2%) and MicroScan (86.9%) systems; however, VITEK MS showed a lower rate of mis-ID (0.6%) than VITEK 2 (10.8%) and MicroScan (13.1%) for these isolates. Instead, VITEK MS yielded more "no ID" results (9.1%) than VITEK 2 and MicroScan (both, 0%). These results suggest that the VITEK MS system can be advantageous in the routine diagnostic procedures conducted in clinical microbiology laboratories, because an isolate not identified by the primary testing method can be routinely retested or tested by using other methods [15, 20]. In addition, the low rate of mis-ID obtained using the VITEK MS system offers an important clinical advantage along with offering more rapid and reliable ID of other bacterial isolates [16].

Although the need for species ID of *Acinetobacter* isolates in routine clinical microbiology laboratories has been questioned, recent studies have highlighted the importance of discriminating the ABG from non-ABG BSI isolates since non-ABG BSI has a more benign clinical course than ABG BSI, and the two groups differ in their antimicrobial susceptibilities [2, 9, 11, 21-23]. Of the 3 systems tested, VITEK MS showed the best discrimination ability between the ABG and non-ABG isolates, with 100% specificity (0% false-positive rate), while the VITEK 2 and MicroScan systems both showed 31.8% specificity (68.2% false-positive rate) for discriminating between these groups. This finding highlights the effectiveness of VITEK MS for discriminating the ABG from non-ABG isolates in *Acinetobacter* BSI cases. Thus, use of VITEK MS will facilitate prediction of the clinical course and outcome of BSI patients as well as the selection of an appropriate antimicrobial regimen.

The current and previous studies have demonstrated that the limitation of the current VITEK MS system for ID of *Acinetobacter* species [15, 16]. It cannot differentiate among the ABG species such as *A. baumannii*, *A. pittii*, and *A. nosocomialis*. However, when a cluster analysis was performed on the *Acinetobacter* isolates tested in this study by using VITEK MS RUO (research-use-only, Saramis database, bioMérieux) mode, the *A. baumannii*, *A. pittii*, and *A. nosocomialis* isolates were relatively well-clustered (data not shown). In addition, a recent report showed that another MALDI-TOF MS-based system that was unable to reliably differentiate between these closely related species has now been improved by using an alternative protocol [24]. Thus, the VITEK MS system shows potential for offering reliable species-level ID of ABG isolates in routine diagnostic procedures conducted in clinical microbiology laboratories.

Authors' Disclosures of Potential Conflicts of Interest

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

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