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Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Can Accurately Differentiate *Aeromonas dhakensis* from *A. hydrophila*, *A. caviae*, and *A. veronii*

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Among 217 *Aeromonas* isolates identified by sequencing analysis of their *rpoB* genes, the accuracy rates of identification of *A. dhakensis*, *A. hydrophila*, *A. veronii*, and *A. caviae* were 96.7%, 90.0%, 96.7%, and 100.0%, respectively, by the cluster analysis of spectra generated by matrix-assisted laser desorption ionization–time of flight mass spectrometry.

Most human infections caused by *Aeromonas* spp. have been associated with three species: *Aeromonas hydrophila*, *A. veronii*, and *A. caviae* (1–4). Recently, increasing evidence has demonstrated that *A. dhakensis* is an important species, which might cause severe soft tissue and bloodstream infections (5, 6). Although clinical infections caused by *A. dhakensis* have been reported in Taiwan (7), this species have been recovered from aquatic environments and clinical samples globally (8, 9). However, the prevalence of clinical infections caused by *A. dhakensis* is underestimated due to the possibility of this species being misidentified as *A. hydrophila* by the phenotype-based identification system (9, 10).

Previous publications suggested that *A. hydrophila* subsp. *dhakensis* and *A. aquariorum* represented the same taxon (11, 12). Although accurate identification of *Aeromonas* species could be achieved using the nucleotide sequences of housekeeping genes (7, 10), these molecular methods are labor-intensive and time-consuming. Recently, several studies have shown that the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) can rapidly and accurately identify different *Aeromonas* species (13–15). However, among aeromonads that are studied using this method, species identification of *A. dhakensis* rarely has been discussed. In the present study, we assess the performance of two commercially available phenotypic identification systems, the Vitek 2 GN card and Phoenix system NMIC/ID-72 cards (Becton, Dickinson Microbiology Systems), and a MALDI-TOF MS system, the MALDI Biotyper system (microflex LT; Bruker Daltonik GmbH, Bremen, Germany), to identify the clinical *Aeromonas* species in Taiwan.

We analyzed a total of 217 nonduplicated clinical isolates of *Aeromonas* obtained from clinical specimens of the patients at the study hospital between 1998 and 2012, as well as 9 reference strains. All the isolates were stored at −70°C until use. Species identification of these clinical isolates was based on the sequence analysis of the partial *rpoB* gene (16). For the MALDI Biotyper system, the samples were prepared as previously described (15). The *rpoB* identification and MALDI-TOF procedures are described in the Materials and Methods in the supplemental material.

A. aquariorum, *A. hydrophila* subsp. *dhakensis*, *A. hydrophila* subsp. *ranae*, *A. sanarellii*, and *A. taiwanensis* are not included in the current database for Vitek 2, the Phoenix system, and the MALDI Biotyper. Reference strains of *A. aquariorum* and *A. hydrophila* subsp. *dhakensis* were classified as *A. hydrophila* in the Vitek 2 and Phoenix systems (Table 1). *A. aquariorum* strain MDC47^T was misidentified as *A. caviae* (score value, 2.058) and *A. hydrophila* subsp. *dhakensis* strain LMG 19562 was identified as *A. hydrophila* (score value, 2.096). The *A. hydrophila* subsp. *ranae* BCRC 17768 strain was identified as *A. hydrophila* (Table 1).

The characteristic spectra generated by the MALDI Biotyper for the six *Aeromonas* species are shown in Fig. S1 in the supplemental material. The accurate identification rates for species listed in the database (BD 5627) by the MALDI Biotyper system were 93.4% (57/61) for *A. veronii*, 97.1% (34/35) for *A. hydrophila*, and 83.9% (56/61) for *A. caviae*. All *A. dhakensis* isolates exhibited positive Voges-Proskauer (VP) but negative L-arabinose reactions. In the case of the Phoenix system, the accurate identification rate was 96.7% for 61 *A. sobria* isolates, 77.1% for 35 isolates of *A. hydrophila*, and 70.5% for 61 isolates of *A. caviae* (Table 2). In contrast, 83.4% (181/217) of the *Aeromonas* isolates were misidentified at the species level by the Vitek 2 system.

The dendrogram obtained from the MALDI Biotyper data of 123 genetically well characterized isolates is shown in Fig. 1, which shows five cluster groups with a default critical distance level of 850. These cluster groups are in accordance with those established

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TABLE 1 Identification results of nine reference strains of *Aeromonas* species by two automatic identification systems and MALDI Biotyper system

Reference *Aeromonas* strain	Results from MALDI Biotyper system		Results from Phoenix system		Results from Vitek 2 system	
	Aeromonas sp.	Score	*Aeromonas* sp.	Identity (%)	*Aeromonas* sp.	Degree of discrimination[a]
A. aquariorum MDC47^T	*A. caviae*	2.085	*A. hydrophila*	99	*A. hydrophila/caviae*	ED
A. hydrophila subsp. *dhakensis* LMG 19562	*A. hydrophila*	2.096	*A. hydrophila*	99	*A. hydrophila/caviae*	ED
A. hydrophila ATCC 7966^T	*A. hydrophila*	2.203	*A. sobria*	96	*A. hydrophila/caviae*	ED
A. hydrophila BCRC 16704	*A. hydrophila*	2.228	*A. hydrophila*	99	*A. hydrophila/caviae*	ED
A. hydrophila BCRC 13881	*A. hydrophila*	2.370	*A. veronii*	97	*A. hydrophila/caviae*	ED
BCRC 17768 (*A. hydrophila* subsp. *ranae* Huys et al., 2003)	*A. hydrophila*	2.358	*A. sobria*	96	*A. hydrophila/caviae*	LD
A. veronii biovar *sobria* ATCC 9071^T	*A. veronii*	2.165	*A. sobria*	99	*A. hydrophila/caviae*	LD
A. caviae ATCC 13136^T	*A. caviae*	2.290	*A. sobria*	94	*A. hydrophila/caviae*	ED
A. bestiarum ATCC 13444	*A. bestiarum*	2.160	*A. veronii*	99	*A. hydrophila/caviae*	ED

[a] ED, excellent discrimination; LD, low discrimination.

by species identification by *rpoB* sequencing, with some variations. *A. dhakensis* was closer to *A. veronii* than other species in the MALDI-TOF dendrogram, with dividing branches linked at a distance level of 700.

The above-mentioned 123 isolates used for dendrogram establishment were analyzed for specific signals by clustering analysis (Fig. 2). Another 100 isolates from four *Aeromonas* species were evaluated for external validation. The accuracy identification rates by MALDI-TOF for *A. dhakensis*, *A. hydrophila*, *A. veronii*, and *A. caviae* were 96.7%, 90.0%, 96.7%, and 100.0%, respectively (Table 3).

Since there is no information regarding *A. dhakensis* in the database, some *A. dhakensis* isolates determined by gene sequencing are identified as *A. hydrophila* or *A. caviae* using the current MALDI-TOF database. The discrimination power for differentiating *A. dhakensis* from other species in the cluster analysis could be increased if the database were updated by the novel spectra of *A. dhakensis* isolates generated from this study.

Nearly all (97.0%) of *Aeromonas* isolates were correctly identified to the species level using the new model we generated. The discrepancy rate (3/100 [3%]) was comparable with the rate of 8.6% (12/139) reported by Lamy et al. (17). The advantages of identifying *Aeromonas* species by MALDI-TOF are enhanced compared with those of using commercial phenotypic systems. In the present study, the concordance rate of Vitek 2 GN and Phoenix NMIC/ID-72 at the species level to MALDI-TOF was 16.6% (36/217) and 59.4% (129/217), respectively. In contrast, in the study by Lamy et al. (17), in which *A. dhakensis* isolates were not included, the concordance rate of Vitek 2 GN and Phoenix NMIC/ID-72 was 82.7% and 73.5%, respectively (17).

TABLE 2 Performances of routine phenotypic identification method and MALDI Biotyper system for 217 clinical isolates of *Aeromonas* species

Aeromonas species identified by gene sequencing (no. of isolates)	Results from MALDI Biotyper system		Results from Phoenix system		Results from Vitek 2 system	
	Species (no. of isolates)	Correct ID[a] (%) to species level (score, ≥2.0)	Species (no. of isolates)	Correct ID (%)	Species (no. of isolates)	Correct ID (%)
A. dhakensis (58)	*A. hydrophila* (52) *A. caviae* (5) *A. jandaei* (1)	0	*A. hydrophila* (48) *A. veronii* (4) *A. sobria* (4) Unidentified organism (2)	0.0	*A. hydrophila/A. caviae* (58)	0
A. hydrophila subsp. *hydrophila* (35)	*A. hydrophila* (34) *A. caviae* (1)	97.1	*A. hydrophila* (27) *A. veronii* (8)	77.1	*A. hydrophila/A. caviae* (35)	100
A. veronii (61)	*A. veronii* (57) *A. ichthiosmia* (4)	93.4	*A. sobria* (59) Unidentified organisms (2)	0	*A. hydrophila/A. caviae* (21) *A. sobria* (34) *A. veronii* (1) *A. sobria/A. veronii* (5)	1.6
A. caviae (61)	*A. caviae* (56) *A. hydrophila* (4) *A. jandaei* (1)	91.8	*A. caviae* (43) *A. hydrophila* (9) *A. veronii* (5) *A. sobria* (2) Unidentified organism (2)	70.5	*A. hydrophila/A. caviae* (61)	0
A. sanarellii (1)	*A. caviae* (1)	0	*A. veronii*	0	*A. hydrophila/A. caviae*	0
A. taiwanensis (1)	*A. caviae* (1)	0	*A. veronii*	0	*A. hydrophila/A. caviae*	0

[a] ID, identification.

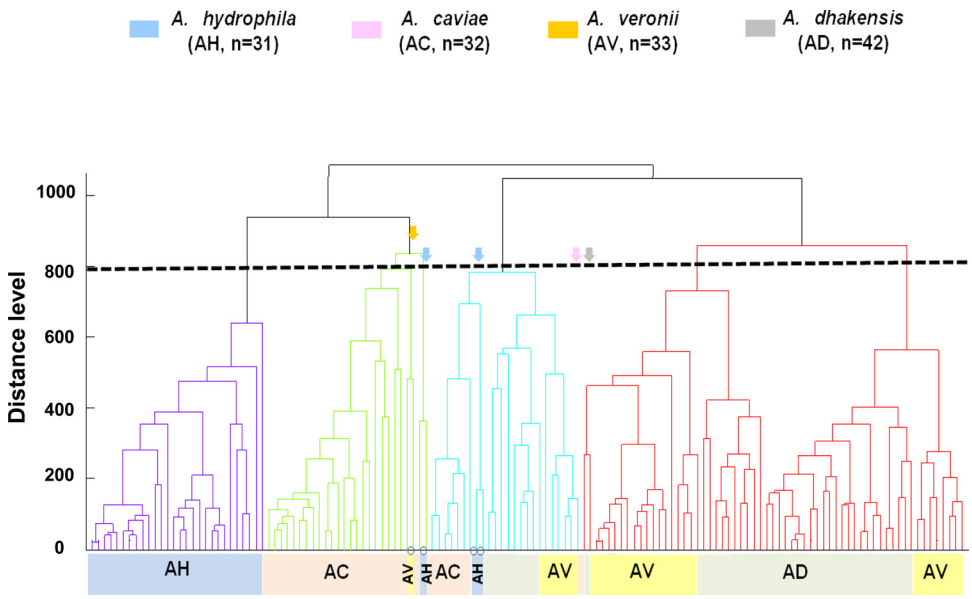

FIG 1 Principal component analysis (PCA) dendrogram generated by MALDI Biotyper mass spectra for 123 isolates of four *Aeromonas* species, including 118 clinical isolates and five reference strains. The four colors indicate the four *Aeromonas* species identified by gene sequencing analysis. The arrows indicate five isolates located in the incorrect clusters of species identified by gene sequencing analysis.

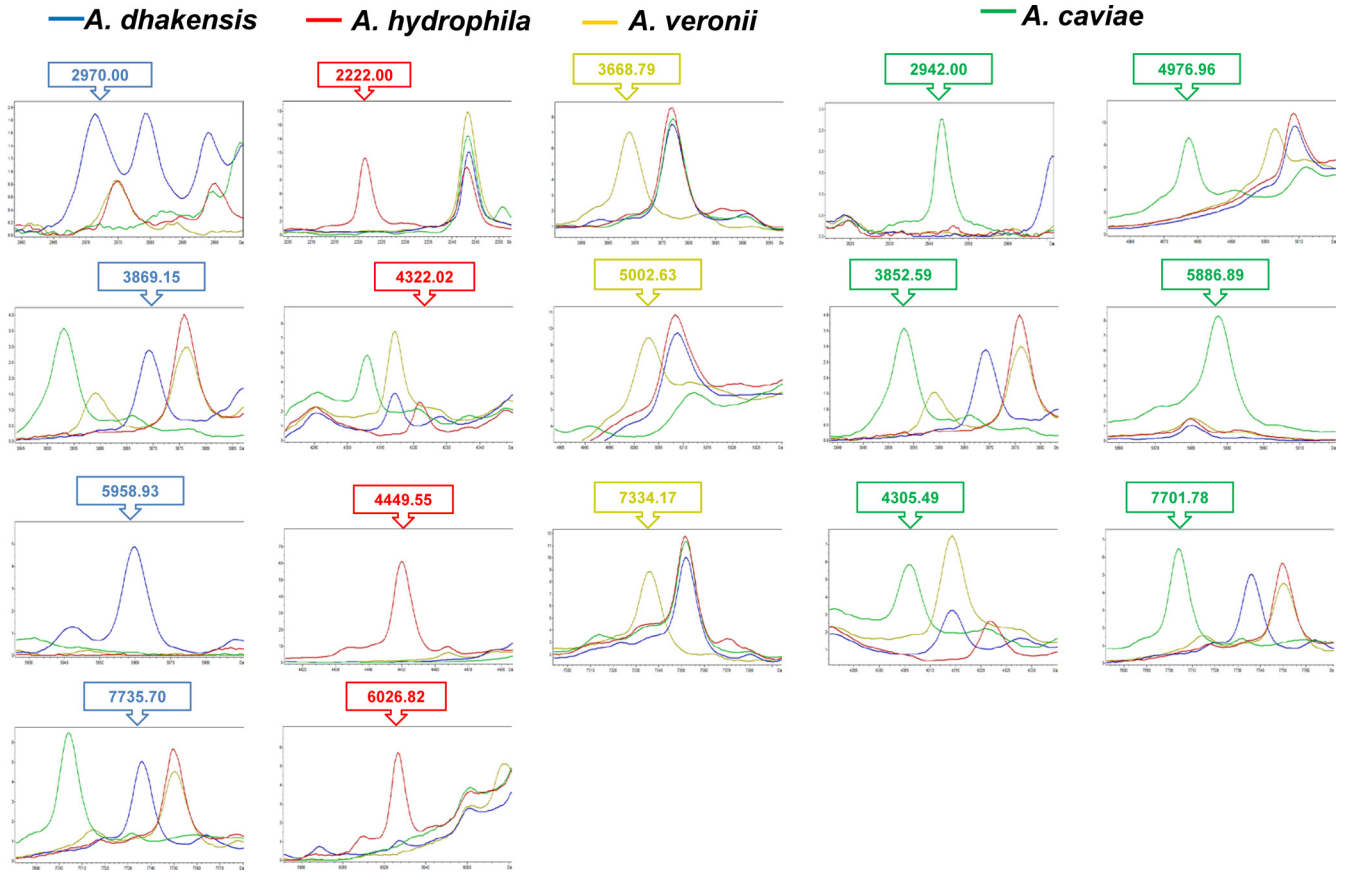

FIG 2 Clustering analysis of MALDI Biotyper system results for four *Aeromonas* species. The signals generated by ClinProTools with the genetic algorithm were specific for identifying varied *Aeromonas* species: 2,970.00, 3,869.15, 5,958.93, and 7,735.70 *m/z* in *A. dhakensis*; 2,222.00, 4,322.02, 4,449.55, and 6,026.82 *m/z* in *A. hydrophila* isolates; 3668.79, 5,002.63, and 7,334.17 *m/z* in *A. veronii*; and 2,942, 3,852.59, 4,305.49, 4,976.96, 5,886.89, and 7,701.78 *m/z* in *A. caviae*.

TABLE 3 Results of external validation of 100 isolates of four *Aeromonas* species by MALDI Biotyper system

Aeromonas species	No. of isolates	No. (%) of isolates with indicated *Aeromonas* species by external validation			
		A. dhakensis	*A. hydrophila*	*A. veronii*	*A. caviae*
A. dhakensis	30	**29 (96.7)**[a]	1 (3.3)		
A. hydrophila subsp. *hydrophila*	10		**9 (90)**	1 (10)	
A. veronii	30	1 (3.3)		**29 (96.7)**	
A. caviae	30	0 (0)			**30 (100)**

[a] Bold type indicates rates of correct identification by external validation results.

The correct identification of *A. dhakensis* among *Aeromonas* isolates is clinically important for optimizing antimicrobial therapy, because a significant proportion of *A. dhakensis* isolates in Taiwan were found to carry resistance genes (18), such as AmpC-like β-lactamase, which is responsible for cephalosporin resistance (19), and *cphA* metallo-β-lactamase, which is responsible for carbapenem resistance (20). The MIC data determined by the Phoenix NMIC/ID-72 system are reported to have a good correlation with the results of the CLSI reference broth microdilution method in *Aeromonas* isolates (21, 22). We found the resistance rates of ertapenem and gentamicin among *A. dhakensis* isolates to be 12.1% and 6.9%, respectively (see Table S1 in the supplemental material), highlighting its potential for antimicrobial resistance.

The dendrogram in Fig. 1 shows the heterogeneity of the protein "fingerprint" in *A. dhakensis* and *A. veronii* isolates. The MALDI-TOF MS method has the potential to group the isolates below the species level. However, the MALDI-TOF dendrogram does not have genetically discriminative information the way other molecular typing methods, such as multilocus sequence typing or pulsed-field gel electrophoresis, do. Further studies comparing the results between MALDI-TOF and other methods at the subspecies level are warranted.

In summary, MALDI-TOF MS might have the potential to be incorporated into the routine microbiology laboratory workflow for rapid identification of aeromonads.

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