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RESEARCH ARTICLE

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Evaluation of three sample preparation methods for the identification of clinical strains by using two MALDI-TOF MS systems

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Abstract

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has revolutionized the microbial identification, especially in the clinical microbiology laboratories. However, although numerous studies on the identification of microorganisms by MALDI-TOF MS have been reported previously, few studies focused on the effect of pretreatment on identification. Due to the sensitivity of MALDI-TOF MS, different preparation methods will lead to changes in microbial protein fingerprints. In this study, for evaluating a more appropriate preparation method for the clinical microbiology identification, we analyzed the performance of three sample preparation methods on two different MALDI-TOF MS systems. A total of 321 clinical isolates, 127 species, were employed in the comparative study of three different sample preparation methods including the direct colony transfer method (DCTM), the on-target extraction method (OTEM), and the in-tube extraction method (ITEM) compatible with MALDI-TOF MS. All isolates were tested on the Microflex LT and Autof ms1000 devices. The spectra were analyzed using the Bruker biotyper and the Autof ms1000 systems. The results were confirmed by 16/18S rRNA sequencing. Results reveal that the accuracies of isolates identification by Bruker biotyper successfully identified 83.8%, 96.0%, and 95.3% after performing the DCTM, OTEM, and ITEM, respectively, while the Autof ms1000 identified 97.5%, 100%, and 99.7%. These data suggested that the identification rates are comparable among the three preparation methods using the Autof ms1000 and Bruker microflex LT systems but the OTEM is more suitable and necessary for clinical application, owing to its key advantages of simplicity and accuracy.

KEYWORDS
clinical microbiology, isolates identification, MALDI-TOF MS, OTEM, sample preparation

Keya Cai and Peijuan Yu contributed equally.

1 | INTRODUCTION

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a new technology for microbial identification.[1,2] It is a useful tool for proteomics analysis with advantages of excellent sensitivity, high throughput, simple operation, and low cost.[3–7] In recent years, it has been widely recognized in the field of microbial identification and played an important role in the rapid detection of clinical microorganisms.[8,9] However, ideal MALDI-TOF MS implementation in clinical microbiology requires every well-established procedure for this technology. Numerous researches have discussed the relative procedures. Most of them usually focus on the comparison of the identification effects of different methodologies or mass spectrometry platforms.[10,11] As we know, appropriate sample pretreatment methods are also one of the important steps for the successful identifications of microorganisms. To date, some papers have been mentioned the distinction of different pretreatment methods. Nevertheless, in consideration of a variety of common or difficult-to-identify microorganisms derived from form clinic, these researches only pay attention on specific species or samples, such as Vibrio species from seafood[12] and anaerobic bacteria from blood.[13] Furthermore, in current clinical microbiology laboratories, there are several routine sample preparation methods with its own advantages and disadvantages. Direct colony transfer method (DCTM) and on-target extraction method (OTEM) as simple pretreatment approaches often used for identifications of common bacteria, such as Gram-negative bacteria, Gram-positive bacteria, and mucinous bacteria, while in-tube extraction method (ITEM) as a more advanced but time-consuming preparation methods is used for difficult-to-identify microorganisms.[14] Since microbiological technical personnel often deal with unknown bacteria in advance, it is very crucial for them to choose efficient method strategy for clinical application. An ideal sample preparation method not only must be met simple, rapid, and robust traits[15,16] but also could be fulfilled the routine clinical experiment and covered common strains. The aim of the present study was to analyze the performance of these common sample preparation methods on two different MALDI-TOF MS systems and evaluate a more appropriate preparation method for rapid identification in a clinical routine microbiology laboratory.

2 | MATERIALS AND METHODS

2.1 | Micro-organism isolates

A total of 321 clinical isolates, comprising aerobes (n = 277), anaerobes (n = 14), and yeasts (n = 30), were included in this study. The clinical isolates were obtained from the Second Affiliated Hospital of Soochow University.

Escherichia coli ATCC25922, *Neisseria meningitides* ATCC13090, *Nocardia asteroides* ATCC19247, *Staphylococcus aureus* ATCC29213, *Haemophilus influenza* ATCC49766, *Clostridium difficile* ATCC9689, and *Candida albicans* ATCC66027 were used as reference strains.

All the isolates were stored at −40°C. Before identification, frozen isolates were subcultured twice under suitable culture conditions to obtain the pure cultures.

2.2 | Sample preparation

Each isolate was performed by three different extraction procedures. All three procedures are derived from the Clinical Laboratory and Standards Institute (CLSI) M58.[17]

2.2.1 | Direct colony transfer method

A single colony was directly smeared onto the target plate and then overlaid with 1 μl α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution (a saturated solution of α-cyano-4-hydroxycinnamic acidin 50/50 [v/v] of acetonitrile/H2O containing 2.5% trifluoroacetic acid[18]; Autobio Diagnostics, Zhengzhou, China). After air drying at room temperature, the samples were sequentially analyzed by using the Bruker biotyper (Bruker Daltonics, Bremen, Germany) and Autof ms1000 (Autobio Diagnostics, Zhengzhou, China) systems.

2.2.2 | On-target extraction method

A single colony was directly smeared onto the target plate, applied with 1 μl of 70% formic acid, dried at room temperature, and then overlaid with 1 μl CHCA matrix solution. After air drying at room temperature, the samples were sequentially analyzed by using the Bruker biotyper and Autof ms1000 systems.

2.2.3 | In-tube extraction method

Several colonies were mixed with 300 μl of distilled water and 900 μl of ethanol in a 1.5 ml Eppendorf tube. The samples were pelleted by centrifuging them at 13,000g for 2 min, dried, and reconstituted in 15 μl of 70% formic acid. After vortexing, 15 μl of acetonitrile was added, and the suspension was centrifuged at 13,000g for 2 min. A total of 1 μl of the supernatant was transferred to the target plate, dried at room temperature, and then overlaid with 1 μl CHCA matrix solution. After air drying at room temperature, the samples were sequentially analyzed by using the Bruker biotyper and Autof ms1000 systems.

2.3 | MALDI-TOF MS identification

After extraction procedures, all the isolates were identified by Bruker biotyper (positive ion, linear mode; ion source voltage 1:20 kv; focusing voltage 7 kv; detector voltage 2.65 Kv; delayed extraction time of 230 ns; detection quality range: 2,000–20,000 Da) and Autof ms1000

(positive ion, linear mode, delayed extraction time of 200 ns, acceleration voltage of 20 kV, and a grid voltage of 93%; detection quality range: 2,000–20,000 Da), followed the manufacturers' instructions. For Bruker biotyper, the spectra data were collected and analyzed by the Bruker Biotyper 3.1 software, library number v5.0 5898 (containing 5,989 MSPs.). The calibration was done using Bacterial Test Standard (BTS) (Bruker Daltonics, Bremen, Germany). The manufacturer's interpretation criteria were applied, with a score ≥2.0 for species-level identification, a score between 1.7 and 2 for genus-level identification, and a score <1.7 for unidentified.

For Autof ms1000, the spectra data were collected by software Autof Acquirer version 1.0.123 and analyzed by software Autof Analyzer version 1.0.50, library number v1.1.0 (containing 9,531 MSPs). The calibration was done using Autobio calibrating agent (Autobio Diagnostics, Zhengzhou, China). The manufacturer's interpretation criteria were applied, with a score >9.0 for species-level identification, a score between 6.0 and 9.0 for genus-level identification, and a score <6.0 for unidentified.

2.4 | Data analysis

By two different MALDI-TOF MS systems, isolates that cannot be identified to the species level or shown different identification results were confirmed by 16/18S rRNA sequencing according to the CLSI MM18-A.[19] The resulting nucleotide sequences were aligned and Basic Local Analysis Search Tool (BLAST) matched against the reference sequences in the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/). The MALDI data were categorized as follows: (i) correct identification (correct identification at the species level and correct identification at the genus level); (ii) no identification (no peaks found or no reliable identification); and (iii) misidentification (wrong identification at the species level.[20]

2.5 | Data statistics

Using graph pad prism 8 software, data were tested by chi square or fisher's exact tests. When $p < 0.05$, the difference was significant.

3 | RESULTS

3.1 | Evaluation of the efficacy of the Bruker biotyper system combined with three different extraction methods

In Table 1, the identification results and extraction methods used for the analysis with the Bruker biotyper system are presented.

Using the DCTM, 83.8% (269/321) of clinical isolates were correctly identified, and 66.7% (214/321) of isolates were accurately identified at the species level. Among the clinical isolates, the correct identification rates of bacteria and yeast isolates were 89.0% (259/291) and 33.3% (10/30), but 72.9% (212/291) and 6.7% (2/30) at the species level. A total of 15.0% (48/321) of isolates were not identified, including 16 Gram-positive bacteria, 12 Gram-negative bacteria, and 20 yeast. A total of 1.2% (4/321) of bacteria isolates were misidentified, including 1 Gram-positive bacteria and 3 Gram-negative bacteria.

Using the OTEM, the higher correct identification rates were achieved. A total of 96.0% (308/321) of clinical isolates were correctly identified, and 84.4% (271/321) of isolates were accurately identified at the species level. Among the clinical isolates, the correct identification rates of bacteria and yeast isolates were 96.2% (280/291) and 93.3% (28/30), but 87.3% (254/291) and 56.7% (17/30) at the species level. A total of 2.8% (9/321) of isolates were not identified, including three Gram-positive bacteria, four Gram-negative bacteria, and two yeast. A total of 1.2% (4/321) of bacteria isolates were misidentified, including one Gram-positive bacteria and three Gram-negative bacteria.

Using the ITEM, 95.3% (306/321) of clinical isolates were correctly identified, and 81.6% (262/321) of isolates were accurately identified at the species level. Among the clinical isolates, the correct identification rates of bacteria and yeast isolates were 95.9% (279/291) and 73.3% (22/30), but 82.3% (240/291) and 73.2% (22/30) at the species level. A total of 3.4% (11/321) of isolates were not identified, including three Gram-positive bacteria, five Gram-negative bacteria, and three yeast. A total of 1.2% (4/321) of bacteria isolates were misidentified, including four Gram-negative bacteria. The data statistics difference was significant among three different extraction methods (Figure 1, left).

3.2 | Evaluation of the efficacy of the Autof ms1000 system combined with three different extraction methods

In Table 2, the identification results and extraction methods used for the analysis with the Autof ms1000 system are presented.

Using the DCTM, 97.5% (313/321) of clinical isolates were correctly identified, and 90.0% (289/321) of isolates were accurately identified at the species level. Among the clinical isolates, the correct identification rates of bacteria and yeast isolates were 98.3% (286/291) and 90.0% (27/30), but 95.2% (277/291) and 40% (12/30) at the species level. A total of 2.5% (8/321) of isolates were not identified, including four Gram-positive bacteria, one Gram-negative bacteria, and three yeasts. No strains were misidentified.

Using the OTEM, 100% (321/321) of clinical isolates were correctly identified, and 98.8% (317/321) of isolates were accurately identified at the species level. Among the clinical isolates, the correct identification rates of bacteria and yeast isolates were 98.6% (287/291) and 100% (30/30) at the species level. No strains were missed or misidentified.

Using the ITEM, 99.7% (320/321) of clinical isolates were correctly identified, and 98.4% (316/321) of isolates were accurately identified at the species level. Among the clinical isolates, the correct

TABLE 1 Identification of 321 clinical isolates using the Bruker biotyper system combined with three different extraction methods

Extraction method	Microorganism (N of isolates)	N(%) of isolates			
		Correct identification			
		Species level	Genus level	No identification	Misidentification
DCTM	Bacteria (291)	212	47	28	4
	Aerobes (277)	203	43	27	4
	Gram-positive (114)	65	32	16	1[a]
	Gram-negative (163)	138	11	11	3[b]
	Anaerobes (14)	9	4	1	-
	Gram-positive (5)	4	1	-	-
	Gram-negative (9)	5	3	1	-
	Yeasts (30)	2	8	20	-
	Total (321)	214 (66.7)	55 (17.1)	48 (15.0)	4 (1.2)
OTEM	Bacteria (291)	254	26	7	4
	Aerobes (277)	241	25	7	4
	Gram-positive (114)	97	13	3	1[c]
	Gram-negative (163)	144	12	4	3[d]
	Anaerobes (14)	13	1	-	-
	Gram-positive (5)	5	-	-	-
	Gram-negative (9)	8	1	-	-
	Yeasts (30)	17	11	2	-
	Total (321)	271 (84.4)	37 (11.5)	9 (2.8)	4 (1.2)
ITEM	Bacteria (291)	240	39	8	4
	Aerobes (277)	227	38	8	4
	Gram-positive (114)	89	22	3	-
	Gram-negative (163)	138	16	5	4[e]
	Anaerobes (14)	13	1	-	-
	Gram-positive (5)	4	1	-	-
	Gram-negative (9)	9	-	-	-
	Yeasts (30)	22	5	3	-
	Total (321)	262 (81.6)	44 (13.7)	11 (3.4)	4 (1.2)

Abbreviations: DCTM, direct colony transfer method; OTEM, on-target extraction method; ITEM, in-tube extraction method.
[a]One isolate of *Bacillus subtilis* was misidentified as *Bacillus mojavensis* by the Bruker biotyper system after the DCTM.
[b]One isolate of *Burkholderia cepacia*, one isolate of *Ochrobactrum anthropi*, and one isolate of *Yersinia kristensenii* were misidentified as *Burkholderia cenocepacia*, *Ochrobactrum intermedium*, and *Yersinia enterocolitica*, respectively, by the Bruker biotyper system after the DCTM.
[c]One isolate of *Paenibacillus polymyxa* was misidentified as *Paenibacillus jamilae* by the Bruker biotyper system after the OTEM.
[d]One isolate of *Burkholderia cepacia*, one isolate of *Serratia marcescens*, and one isolate of *Yersinia kristensenii* were misidentified as *Burkholderia cenocepacia*, *Serratia ureilytica*, and *Yersinia enterocolitica*, respectively, by the Bruker biotyper system after the OTEM.
[e]Two isolates of *Serratia marcescens* were misidentified as *Serratia ureilytica*, and one isolate of *Burkholderia cepacia* and one isolate of *Yersinia kristensenii* were misidentified as *Burkholderia cenocepacia* and *Yersinia enterocolitica*, respectively, by the Bruker biotyper system after the ITEM.

identification rates of bacteria and yeast isolates were 98.3% (286/291) and 100% (30/30) at the species level. One Gram-negative bacteria strain was misidentified. The data statistics difference was significant among three different extraction methods (Figure 1, right).

4 | DISCUSSION

MALDI-TOF MS is considered as an important technology in clinical microbiological laboratories.[21–23] Appropriate pretreatment before analysis can make identification of microorganisms more efficient, rapid, and accurate.[24] In this study, we evaluated the performance of the three sample preparation methods (DCTM, OTEM, and ITEM) in the identification of 321 clinical isolates by two different MALDI-TOF MS systems, using 16/18S rRNA gene sequencing as the reference method. Overall, after the three extraction procedures, more than 80% of isolates could be correctly identified by the two systems. And it is worth mentioning that the OTEM is an appropriate preparation method to embrace better identification accuracy, considering that the ITEM is more time-consuming and laborious.

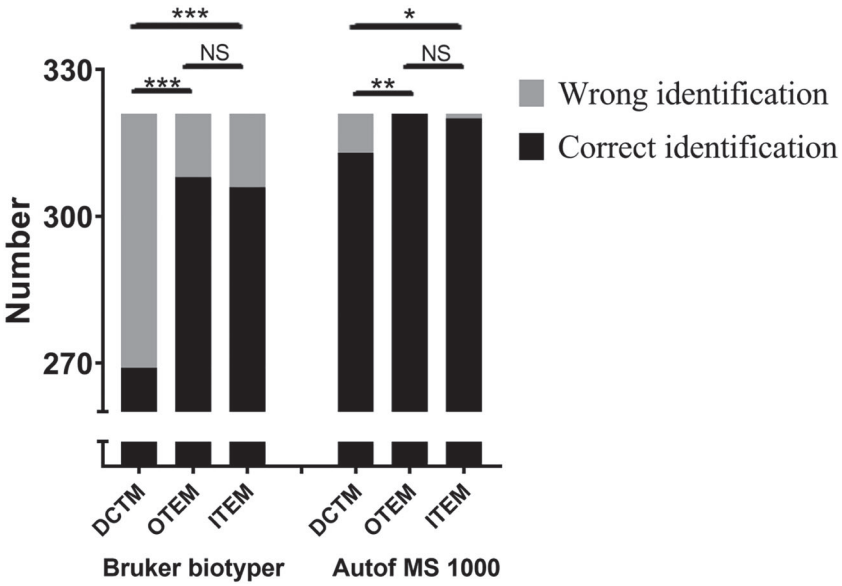

FIGURE 1 Identification results on the Autof ms1000 and Bruker Biotyper. NS, not significant; two-tailed p values < 0.05*; p < 0.01**; p < 0.001***

Numerous studies on the identification of microorganisms by MALDI-TOF MS have been reported previously, but the effect of pretreatment on identification is often neglected.[10,25,26] Due to the sensitivity of MALDI-TOF MS, different pretreatment methods will lead to changes in microbial protein fingerprints.[27] Therefore, the pretreatment is the most important link affecting the identification results. Typically, the method strategy that the microbiological technical personnel adopt is based on their direct experience or SOPs (e.g., treated common Gram-negative bacteria with DCTM; Gram-positive bacteria with OTEM; and treated mucinous bacteria, difficult-to-identify bacteria, yeasts, etc. with ITEM[9]). However, this mode may cause daily heavy burden on clinical microbiology laboratories due to repeated identification work, resulting in a waste of time, personnel, supplies, and other costs. If the microbiological personnel can select an appropriate preparation method, the identification efficiency and accuracy rate of MALDI-TOF MS will be improved.

It is clear that the pretreatment of the direct colony transfer method (DCTM) is quick and easy to operate, but it only can reliably identify microorganisms that are commonly isolated in clinical microbiological laboratories. It is not suitable for identification of fungi and some difficult strains, such as certain Gram-positive bacteria, Mycobacterium spp., and filamentous fungi.[28] In addition, these analyses processed by this method may not release to adequately prepare proteins for detection by MALDI TOF MS. In our study, the total identification accuracy of 321 strains treated with the DCTM by the Bruker Biotyper and the Autof ms1000 system was 83.8% and 97.5%. Similarly, 93% (408/440) Gram-Negative Bacilli isolates were correctly identified by use of the DCTM from Saffert's study on the Bruker Biotyper system.[29] However, another study showed that 56% (168/305) of Gram-Positive Cocci isolates were correctly identified by using the DCTM.[30]

Compared with DCTM, the OTEM increased the additional formic acid pyrolysis process. In our study, the total identification accuracy of 321 strains treated with the OTEM by the Bruker Biotyper and the Autof ms1000 system was 96.0% and 100%. According to a previous research on the Bruker biotyper system, the analysis with the OTEM also showed an excellent correct identification rate 89.0% for all 309 clinical yeast isolates.[31] However, the results contradict an earlier report, in which one, formic acid treatment did not result in a statistically different rate of identification than the direct-smear group (64.6% vs. 64.1%, respectively), for 560 Gram-negative bacteria.[32] This may be due to insufficient database entries that would enable spectral matches.

In our experience, the ITEM is cumbersome for laboratory technologists and results in waste production and high cost. However, strains that cannot be identified with the DCTM will be treated with the ITEM, generally, such as Gram-Positive bacilli and yeast. In our study, the total identification accuracy of 321 strains treated with the ITEM by the Bruker Biotyper and the Autof ms1000 system was 95.3% and 99.7%. These results were not significantly different from that of the OTEM. Similarly, in an earlier study, for 90 yeast and 78 Corynebacterium species isolates, the OTEM yielded identification percentages similar to those for the conventional but more laborious ITEM, 95.6% and 87.8% of yeast and 96.1% and 98.7% of Corynebacterium isolates correctly identified to the genus level, respectively.[33] Moreover, in Alatoom's study, the ITEM resulted in more high level identifications than did DCTM.[30] These studies are consistent with our conclusions.

This study is the first to compare the identification performance of three different sample preparation methods, based on two MALDI-TOF MS systems (Bruker Biotyper and Autof ms1000). In this study, 321 clinical strains were selected, including 277 aerobes, 14 anaerobes, and 30 yeasts, covering Enterobacteriaceae, Staphylococcus, Streptococcus, Enterococcus, Corynebacterium, Candida, and Cryptococcus. After the analysis, two MALDI-TOF MS systems showed the same results for the three different sample preparation methods for all 321 isolates. The results of our study demonstrated that, for the both MALDI-TOF MS systems, the analysis with the

TABLE 2 Identification of 321 clinical isolates using the Autof ms1000 system combined with three different extraction methods

Extraction method	Microorganism (N of isolates)	N (%) of isolates			
		Correct identification			
		Species level	Genus level	No identification	Misidentification
DCTM	Bacteria (291)	277	9	5	-
	Aerobes (277)	263	9	5	-
	Gram-positive (114)	104	7	4	-
	Gram-negative (163)	159	2	1	-
	Anaerobes (14)	14	-	-	-
	Gram-positive (5)	5	-	-	-
	Gram-negative (9)	9	-	-	-
	Yeasts (30)	12	15	3	-
	Total (321)	289 (90.0)	24 (7.5)	8 (2.5)	-
OTEM	Bacteria (291)	287	4	-	-
	Aerobes (277)	273	4	-	-
	Gram-positive (114)	112	2	-	-
	Gram-negative (163)	161	2	-	-
	Anaerobes (14)	14	-	-	-
	Gram-positive (5)	5	-	-	-
	Gram-negative (9)	9	-	-	-
	Yeasts (30)	30	-	-	-
	Total (321)	317 (98.8)	4 (1.2)	-	-
ITEM	Bacteria (291)	286	4	-	1
	Aerobes (277)	272	4	-	1
	Gram-positive (114)	112	2	-	-
	Gram-negative (163)	160	2	-	1[a]
	Anaerobes (14)	14	-	-	-
	Gram-positive (5)	5	-	-	-
	Gram-negative (9)	9	-	-	-
	Yeasts (30)	30	-	-	-
	Total (321)	316 (98.4)	4 (1.2)	-	1 (0.3)

Abbreviations: DCTM, direct colony transfer method; OTEM, on-target extraction method; ITEM, in-tube extraction method.
[a]One isolate of *Burkholderia cepacia* was misidentified as *Burkholderia cenocepacia* by the Autof ms1000 system after the ITEM.

DCTM yielded a lower rate of correct identification than the other two methods, and the analysis with the OTEM showed comparable rates of correct identification than the ITEM method for all isolates. These results suggest that the time- and labor-saving OTEM is more suitable and necessary for the clinical microbiology laboratory, owing to its key advantages of simplicity and accuracy.

Notably, Our results showed that, for the Bruker biotyper system, some of isolates were misidentified, specifically, *Bacillus subtilis* misidentified as *Bacillus mojavensis*, *Burkholderia cepacia* misidentified as *Burkholderia cenocepacia*, *Ochrobactrum anthropi* misidentified as *Ochrobactrum intermedium*, *Yersinia kristensenii* misidentified as *Yersinia enterocolitica*, *Serratia marcescens* misidentified as *Serratia ureilytica*, and *Paenibacillus polymyxa* misidentified as *Paenibacillus jamilae*. For the Autof ms1000 system, only one isolate of *Burkholderia cepacia* was misidentified as *Burkholderia cenocepacia*.

Misidentification by mass spectrometry has also been reported in previous studies. The most typical case of misidentification is that between *Escherichia coli* and *Shigella species*.[34,35] *Escherichia coli* and *Shigella speciesare* very closely related Gram-negative bacteria belonging to the family *Enterobacteriaceae*. They have very similar protein types, and their spectra performance is extremely consistent. Until recently, it has yet been difficult to discriminate them by existing MALDI-TOF MS assays. Considering the limitations of MALDI-TOF MS, the identification of *Escherichia coli* and *Shigella* species will still require additional tests according to the nature of the sample, such as biochemical tests, Clin Pro Tools analysis,[36,37] and other methods. Although small differences in intensity and some minor mass variation were observed from different strains, the highly similar spectra hamper a rapid and straightforward identification and misidentification, occurring

frequently in routine diagnostic laboratories. In addition, other strains are difficult to completely distinguish by MALDI-TOF MS including the *Burkholderiacepacia complex*,[38] the *Acinetobacter baumanni* group,[39] *Streptococcus pneumoniae* and *Streptococcus mitis/oralis*,[40] the *Raoultella spp*,[41] and so on.[42–44] In this study, *Bacillus subtilis* was misidentified as *Bacillus mojavensis*, and on the contrary, in Huang's study, *Bacillus mojavensis* was misidentified as *Bacillus subtilis*.[45] It is possible that the mass spectra of the two bacteria are very similar, and the systematic error, algorithm of the instrument itself, or the microbial database may lead to the final misidentification. Furthermore, there is no report of misidentification about the *Yersinia kristensenii*, *Serratia marcescens*, and so on. Taking H17 as an example, according to CLSI MM18-A, the sample H17 was sequenced by 16S rRNA, matched with the NCBI database, and confirmed to be a *Serratia marcescens*. However, one of the three repeated targets of the same pretreated sample was found to have the wrong identification result by the Bruker Biotyper system. Specifically, the H2 target was misidentified as *Serratia ureilytica*. But by comparison and analysis of the Spectra, there is almost no difference between the two maps (Figure 2). This may be related to instrument stability or database algorithm. Autof ms1000 does not have this situation (Figure 3).

In conclusion, this study demonstrated the performance of three sample preparation methods in identifying numerous clinical isolates, by using two different MALDI-TOF MS systems, and evaluated the OTEM as a best extraction procedure for the routine clinical microbiology. However, this study focused on the clinical common strains, only a small number of Mycobacteria and other difficult strains, and Filamentous fungi were not included. According to previous studies, results for these strains were not encouraging; hence, improved sample extraction protocols and upgraded databases are urgently needed.[46–51]

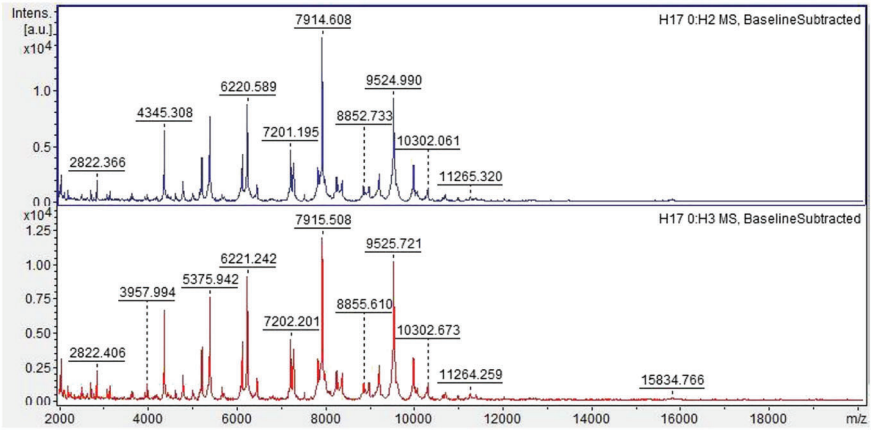

FIGURE 2 Two mass spectra (A and B) obtained at two different spots (H2 and H3) with a single pretreated sample acquired on the Bruker Biotyper system. The identification result of H2 spot was *Serratia ureilytica*, while H3 spot was *Serratia marcescens*

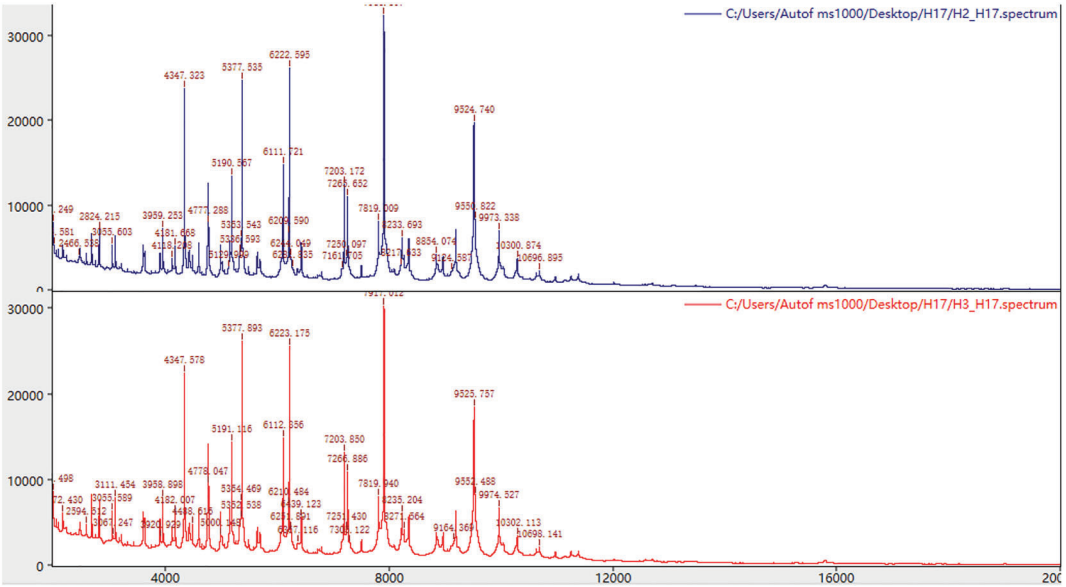

FIGURE 3 Two mass spectra (A and B) obtained at two different spots (H2 and H3) with a single pretreated sample acquired on the Autof ms1000 system. The identification results of H2 and H3 spots were both *Serratia marcescens*

CONFLICT OF INTEREST

There is no conflict of interest in the outcome of this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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