

Comparison of Autof Ms1000 and EXS3000 MALDI-TOF MS Platforms for Routine Identification of Microorganisms

Lijuan Xiong^{1,*}, Xu Long^{1,*}, Lijun Ni^{2,*}, Lili Wang², Yang Zhang¹, Lili Cui¹, Jian Guo², Chunying Yang¹

¹Department of Laboratory Medicine, the Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, Guizhou, People's Republic of China; ²Department of Laboratory Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Jian Guo, Department of Laboratory Medicine, Shanghai East Hospital, Tongji University School of Medicine, No. 1800 Yuntai Road, Pudong New Area, Shanghai, People's Republic of China, Tel +86-17701810639, Email guojian1110@126.com; Chunying Yang, Department of Laboratory Medicine, the Second Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, No. 83 Feishan Street, Yunyan District, Guiyang City, Guizhou Province, People's Republic of China, Tel +86-13658504875, Email 2590470591@qq.com

Purpose: Matrix-assisted laser desorption-ionization-time of flight mass spectrometry (MALDI-TOF) has recently been widely used in clinical microbiology laboratories, with the advantages of being reliable, rapid, and cost-effective. Here, we reported the performance of two MALDI-TOF MS instruments, EXS3000 (Zybio, China) and Autof ms1000 (Autobio, China), which are commonly used in clinical microbiology field.

Methods: A total of 209 common clinical common isolates, including 70 gram-negative bacteria strains, 58 gram-positive bacteria strains, 33 yeast strains, 15 anaerobic bacteria strains, and 33 mold strains, and 19 mycobacterial strains were tested. All strains were identified by EXS3000 (Zybio, China) and Autof ms1000 (Autobio, China). Sequence analysis of 16S rRNA or ITS regions was used to verify all strains.

Results: Current study found that species-level discrimination was found to be 191 (91.39%) and 190 (90.91%) by EXS3000 and Autof ms1000, respectively. Genus-level discrimination was 205 (98.09%) by the EXS3000 and 205 (98.09%) by the Autof ms1000, respectively. The correct results at species level of the EXS3000 were 91.43% (64/70) for gram-negative bacteria, 93.1% (54/58) for gram-positive cocci, 93.94% (31/33) for yeast, 100% (15/15) for anaerobes and 81.82% (27/33) for filamentous fungi. The correct results at species level of the Autof ms1000 were 92.86% (65/70) for gram-negative bacteria, 91.38% (53/58) for gram-positive cocci, 93.94% (31/33) for yeast, 100% (15/15) for anaerobes and 78.79% (26/33) for filamentous fungi.

Conclusion: Although the results show that the EXS3000 and Autof ms1000 systems are equally good choices in terms of analytical efficiency for routine procedures, the test result of EXS3000 is slightly better than Autof ms1000. It's worth mentioning that the target plate of the EXS 3000 instrument is reusable, but the target plate of the Autof ms1000 is disposable, making the EXS3000 more effective in reducing costs.

Keywords: evaluation, MALDI-TOF mass spectrometry, EXS3000, Autof ms1000, clinical isolates, identification, gram-negative bacteria, gram-positive bacteria, mycobacteria, yeast, anaerobic bacteria

Introduction

Over the past two decades, Laboratory diagnosis of melioidosis has shifted to PCR-based sensitive detection methods such as gene sequencing and qPCR, and rapid specific identification techniques such as matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.^{1,2} As a new generation of microbial rapid identification and analysis technology, mass spectrometry is a method to detect proteins or nucleic acid ions with different mass-to-charge ratios after separation by the electric field. This method greatly promotes the development of microbial identification technology.^{3,4} This new application has proven useful in the diagnosis and treatment of infectious diseases that require

a swift response. Identifying microorganisms using MALDI-TOF MS is convenient, rapid, and accurate while also being cost-effective. Thus, this method has revolutionized microbial identification in clinical microbiology laboratories.^{5,6}

Currently, many companies are producing or selling commercial mass spectrometry for clinical microbial identification, such as: Brooker-Dalton, Biomeerier, Antu Biological, Zybio, and so on. It is worth mentioning that Antu Biological Company and Zybio Company are both in vitro diagnostic companies from China. In the past few years, the mass spectrometry manufacturers are basically from Western countries, and in recent years, we began to see mass spectrometry from China on the market. The EXS3000 is a MALDI-TOF MS instrument received CE-marked IVD clearance in October 2021. The Autof ms1000 is a MALDI-TOF MS instrument developed in April 2018 that received CE-marked IVD clearance in June 2018.

These two types of mass spectrometry made in China are from one of the earliest mass spectrometry research and development companies, known as Xiamen Mass Spectrometry. At present, more than 80% of new installed users are occupied by these two manufacturers in the Chinese market.

Considering the nature of bacterial infections and the high mortality rate, rapid diagnosis is necessary for appropriate targeted treatment. Therefore, rapid detection methods are increasingly being valued by clinical Medicine, and mass spectrometry detection methods can fully meet people's needs in this regard.

This study aims to evaluate the ability of MALDI-TOF MS for the identification of microorganisms and compare the diagnostic performance of the Autof ms1000 (Autobio, China) with the EXS3000 (Zybio, China). The identification performance was evaluated in preserved strains and clinical isolates, and the analytical accuracy was compared.

Materials and Methods

Evaluated Microorganisms

This study conforms to the Declaration of Helsinki. The performance evaluation used routine clinical isolates. Clinical isolates were collected from routine specimens received from 1 February 2020 to 31 June 2020 in Shanghai East Hospital. The clinical isolates were derived from frozen strains, and the blood culture positive strains were derived from new blood vial. A total of 209 clinical isolates were isolated from genitourinary, respiratory, blood, gastrointestinal; and other specimens. There were 70 gram-negative bacteria strains, 58 gram-positive bacteria strains, 33 yeast strains, 15 anaerobic bacteria strains, and 33 mold strains. All strains were identified by Zybio MS and Antuf MS. All strains were verified by sequencing analysis.

MALDI-TOF MS Analysis

The laboratory, standard and clinical strains were taken out from the -80°C refrigerator and subcultured twice to obtain fresh cultures. Yeast, filamentous fungi, aerobic bacteria and anaerobic bacteria were each inoculated on suitable medium. The direct transfer method and the formic acid (FA) method followed by ethanol extraction methods were used for bacteria and fungi respectively. Sequence analysis of 16S rRNA or ITS regions was used to verify all strains.

Zybio EXS3000

All procedures were carried out following instrument manual. Mass spectrometry system sample processing matrix solution, mass spectrometry system blood culture positive sample pretreatment kit, mass spectrometry system sample pretreatment solution, calibration product ATCC25922 freeze-dried powder were used in the experiment. The test was performed using EXS3000 (Zybio, China), and EXS3000 software were used to identify the results. *Escherichia coli* ATCC 25922 is used for daily calibration of the instrument. The corresponding 96-well target plate can be reused, which greatly reduces the cost of testing. However, the target version of Autof ms1000 is disposable. The direct transfer procedure is as follows: smear a single colony directly onto the target plate, add $1\mu\text{L}$ the matrix solution (α -cyano-4-hydroxycinnamic acid, CHCA) to the target plate, and wait it to dry at room temperature.

The FA method is as follows: smear a single colony directly onto the target plate, add $1\mu\text{L}$ 70% FA and wait it to dry, apply $1\mu\text{L}$ matrix solution (α -cyano-4-hydroxycinnamic acid, CHCA) and wait it to dry.⁷

All procedures were performed according to the manufacturer's instructions.

The pretreatment procedures for the positive blood culture samples are as follows. First, take a positive blood culture bottle and shake well, use a sterile syringe to draw 1.0 mL culture solution into a 1.5mL centrifuge tube. Add 200µL of lysis buffer with a pipette into the sample and mix well, let it stand at room temperature for 3–5 minutes. Centrifuge at 500 g for 10 minutes, discard the supernatant, add 500µL washing buffer I, mix well and centrifuge for 1 min at 12,000 rpm, discard the supernatant and add 500µL washing buffer II. After mixing thoroughly, centrifuge for 1 min at 12,000 rpm, fully remove the supernatant, add 15–25µL 70% FA according to the separated bacteria amount, then add an equal volume of acetonitrile, mix them, centrifuge for 1 min at 13,000 rpm, add 1µL supernatant onto the target plate, dry it and cover it with 1µL matrix solution and wait it to dry.

The separating gel blood coagulation tube method is as follows. Use a sterile syringe to draw 4 mL of the culture solution from the positive blood culture bottle to the separation gel coagulation tube, centrifuge at 3000 rpm at room temperature for 10 minutes, discard the supernatant, add 1 mL of sterile physiological saline to the off-white precipitate on the upper edge of the separation gel, and mix well. Transfer to a 1.5 mL centrifuge tube, centrifuge at 12,000 rpm for 2 min, discard the supernatant, wash twice until it is clear, add 300µL sterile normal saline and 900µL absolute ethanol to the precipitate and mix well, centrifuge at 12,000 rpm for 2 min, discard the supernatant, add 50µL 70% FA centrifuge at 12,000 rpm for 2 min, take 1µL of supernatant (bacterial protein) onto the target plate for detection.

Autobio Autof Ms1000

All procedures were performed according to the manufacturer's instructions. Automatic microbial mass spectrometry detection system with quality control products, mass spectrometry sample processing matrix solution, mass spectrometry sample pretreatment reagent, mass spectrometry blood culture microbial pretreatment reagent were used in the experiment. Mass spectrometry was performed using a Autobio Autof ms1000, and Acquirer 1.0.151 were used to analyze the results. The database was consistent with Zybio EXS3000. Internal quality control was performed with *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and a blank spot. The target plate of Autof ms1000 is disposable and has 96 spots. The procedures of Autof ms1000 in the direct transfer method, FA extraction method were similar to those of EXS3000.

Comparison of the Identification Results

The consistency of results was compared at the species/complex level and the genus level. Due to the limitations of mass spectrometry for microbial identification, it is considered accurate for any of the complex groups/close relatives. For EXS3000 (Zybio, China), the result evaluation method is based on the manufacturer's regulations: a score of 2.00 or higher as "reliable species level identification"; a score between 1.70 and 1.99 as "reliable genus level identification"; a score of less than 1.70 as "no reliable identification". The Autof ms1000 (Autobio, China) identification results range from 9.000 to 10.000 for "reliable species level identification"; range from 6.000 to 8.999 for "reliable genus level identification; range less than 6.000 for "no reliable identification".

Molecular Identification of Discrepant Results

All strains were verified by sequencing analysis. 16S rRNA sequencing was performed on bacteria, and internal transcribed spacer (ITS) regions were sequenced on fungi. The primers used to amplify the 16S rRNA gene of the isolate are 5'-AGAGTTTGATCMTGGCTCAG-3' (27F) and 5'-TACGGYTACCTTGTACGACTT-3' (1492R), and the primers used for fungi are 5'-TCCGTAGGTGAACCTGCGG-3' (ITS1) and 5'-TCCTCCGCTTATTGATATGC-3' (ITS4). Use BLAST software (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to compare the sequence with the sequence in the GenBank database and explain it according to the CLSI guidelines.⁸

Statistical Analysis

We classified the results by reliable species level identification, reliable genus level identification, false positives, species-level detection rate, genus-level detection rate, unreliable ratio, and error rate, and compared the two instruments for common clinical strains. Identification level. All strains were verified by sequencing analysis. $P < 0.05$ was considered statistically significant, if $P > 0.05$ was not statistically significant. Statistical analyses were conducted using SPSS software version 20.0 (China).

Results

Identification results in Clinical Strains

The MALDI-TOF MS identification results of the EXS3000 and Autof ms1000 for clinical strains are listed in Table 1. The identification performance of 209 clinical strains was evaluated. Including 70 gram-negative bacteria, 58 gram-positive bacteria, 15 anaerobic bacteria and 66 fungi. The detail identification results for gram-negative bacteria are listed in Table S1, gram-positive bacteria in Table S2, fungi in Tables S3 and S4, anaerobic bacteria in Table S5.

For the EXS3000, species-level identification of gram-negative clinical strains achieved 64 (91.43%), genus-level identification for 4 strains, and no reliable results for 2 strains. Species-level identification of gram-positive strains for 54 (93.10%), genus-level identification for 4 strains, and the genus of unreliable strains is zero. The correct rate of Fungus (both yeast and filamentous fungi) identification is 58 (87.88%), 6 and 2 for species-level identification, genus-level identification and no reliable results identification strains respectively. Species-level identification of anaerobes clinical strains achieved 15 (100%), the genus-level and no reliable results both for 0 strains. For the Autof ms1000, species-level identification of gram-negative clinical strains achieved 65 (92.86%), genus-level identification for 4 strains, and no reliable results for 1 strain. Species-level identification of gram-positive strains for 53 (91.38%), genus-level identification for 3 strains, and the genus of unreliable strains is 2. The correct rate of Fungus identification is 57 (86.36%), 8 and 1 for species-level identification, genus-level identification and no reliable results identification strains respectively. Species-level identification of anaerobes clinical strains achieved 15 (100%), the genus-level and no reliable results both for 0 strains. The mass spectrometry identification results and scores of each specimen are shown in Tables 1 and 2.

The 14 genus results for EXS3000 including 14 genus-level identification strains of gram-negative (n = 4), gram-positive (n = 4), filamentous fungi (n = 4), yeast (n = 2) and 4 no reliable identification strains of gram-negative bacteria and *filamentous fungi*. For the Autof ms1000, the 19 nonspecies results including 15 genus-level identification strains from gram-negative (n = 4), gram-positive (n = 3), filamentous fungi (n = 6), yeast (n = 2) and 4 no reliable identification strains from gram-negative, gram-positive and filamentous fungi. However, for the identification of anaerobic bacteria, both instruments have reached a 100% level of identification.

Table 1 Identification Results of Clinical Isolates

Group of Organisms	N	EXS 3000			Autof ms1000		
		Species (%)	Genus (%)	No Reliable ID (%)	Species (%)	Genus (%)	No Reliable ID (%)
Total	209	P=0.86328>0.05					
Gram-negative	70	64(91.43)	4	2	65(92.86)	4	1
Gram-positive	58	54(93.10)	4	0	53(91.38)	3	2
Yeast	33	31(93.94)	2	0	31(93.94)	2	0
Filamentous fungi	33	27(81.82)	4	2	26(78.79)	6	1
Anaerobes	15	15(100.00)	0	0	15(100.0)	0	0
Total	209	191 (91.39)	14	4	190 (90.91)	15	4

Table 2 Comparison of Identification Performance of Two Instruments

Species	N	EXS3000				Autof ms1000			
		ID Species	ID Genus	No ID	MisID Species	ID Species	ID Genus	No ID	MisID Species
Gram-negative bacteria (%)	70	64(91.43)	4(5.71)	2(2.86)	0(0.0)	65(92.86)	4(5.71)	1(1.43)	0(0.0)
Gram-positive cocci (%)	58	54(93.10)	4(6.90)	0(0.0)	0(0.0)	53(91.38)	3(5.17)	2(3.45)	0(0.0)
Yeast (%)	33	31(93.94)	2(6.06)	0(0.0)	0(0.0)	31(93.94)	2(6.06)	0(0.0)	0(0.0)
Anaerobes (%)	15	15(100.0)	0(0.0)	0(0.0)	0(0.0)	15(100.0)	0(0.0)	0(0.0)	0(0.0)
Filamentous fungi (%)	33	27(81.82)	4(12.12)	2(6.06)	0(0.0)	26(78.79)	6(18.18)	1(3.03)	0(0.0)
Total (%)	209	191(91.39)	14(6.70)	4(1.91)	0(0.0)	190(90.91)	15(7.18)	4(1.91)	0(0.0)

Comparison of Identification Performance in Clinical Isolates

We can compare the overall identification performance of the two instruments through [Table 2](#). For EXS3000 correctly identified 191 strains at species-level and 14 strains at genus-level. The Autof ms1000 correctly identified 190 and 15 strains at the species-level and genus-level respectively. Obviously, there is no significant difference in the ability of the two instruments at the species level and the genus level.

The EXS3000 and Autof ms1000 both cannot identify 4 strains, they are 2 strains of gram-negative bacteria and 2 fungi for EXS3000, 1 strain of gram-negative bacteria, 2 strains of gram-positive cocci and 1 strain of fungi for Autof ms1000. The correct results at species level of the EXS3000 were 91.43% (64/70) for gram-negative bacteria, 93.10% (54/58) for gram-positive cocci, 93.94% (31/33) for yeast, 100% (15/15) for anaerobes and 81.82% (27/33) for filamentous fungi. The correct results at species level of the Autof ms1000 were 92.86% (65/70) for gram-negative bacteria, 91.38% (53/58) for gram-positive cocci, 93.94% (31/33) for yeast, 100% (15/15) for anaerobes and 78.79% (26/33) for filamentous fungi.

Identification Results of Mycobacterium Between Two Instruments

We also use mycobacterium strains to evaluate identification performance of these two instruments, including 19 different preserved strains, each strain tested 3 times and 57 sets of comparison data were obtained. The results of two instruments are summarized in [Table 3](#). The detail identification results for Mycobacterium are listed in [Table S6](#). The EXS3000 achieved species-level identification for 43 (75.44%) strains, genus-level and no reliable results identification for 5 (8.77%) and 8 (14.04%) strains, respectively. The Autof ms1000 attain species-level identification for 39 (68.42%) strains and genus-level identification for 5 (8.77%), and no reliable results for 1 (1.75%). However, the misidentification result is 1 (1.75%) and 12(21.06%) strains for EXS3000 and Autof ms1000 respectively.

Comparison of Identification Performance of Clinical Isolates from Blood Culture Kit and Separate Gel Blood Clotting Tube

Identification performance of the two instruments is evaluated by the test results of sample which from blood culture kit and separate gel blood clotting tube. In this experiment, 85 bacterial strains from blood culture bottles were treated with a blood culture kit and separated gel clot method and then used for identification performance test. The results of two instruments are summarized in [Table 4](#). Comparing the overall identification performance results shows that, for blood culture kit sample, the EXS3000 correctly identified 77 (90.59%) strains at the species level and 2 strains at the genus level, the Autof ms1000 correctly identified 74 (87.06%) strains at species level and 4 strains at the genus level. By comparison, we found that EXS 3000 is slightly better than Autof ms1000 in species-level identification, and there is no

Table 3 Comparison of Identification Performance of Mycobacterium

Species	N	Number of Tests	EXS3000				Autof ms1000			
			ID Species	ID Genus	No ID	MisID Species	ID Species	ID Genus	No ID	MisID Species
<i>Mycobacterium abscessus</i>	3	9	0	4	5	0	7	2	0	0
<i>Mycobacterium fortuitum</i>	6	18	18	0	0	0	18	0	0	0
<i>Mycobacterium goodnae</i>	1	3	0	0	3	0	0	0	1	2
<i>Mycobacterium intracellulare</i>	5	15	15	0	0	0	7	1	0	7
<i>Mycobacterium mageritense</i>	1	3	3	0	0	0	3	0	0	0
<i>Mycobacterium simiae</i>	3	9	7	1	0	1	4	2	0	3
Total(%)	19	57	43(75.44)	5(8.77)	8(14.04)	1(1.75)	39(68.42)	5(8.77)	1(1.75)	12(21.06)

Abbreviations: ID, identification; MisID, misidentification.

Table 4 Comparison of Identification Performance of Clinical Isolates from Blood Culture Kit and Separate Gel Blood Clotting Tube

Species	N	EXS3000				Autof ms1000			
		ID Species	ID Genus	No ID	MisID Species	ID Species	ID Genus	No ID	MisID Species
Blood culture kit (%)	85	77(90.59)	2(2.35)	6(7.06)	0(0.0)	74(87.06)	4(4.71)	7(8.24)	0(0.0)
Separate gel blood clotting tube (%)	85	78(91.76)	3(3.53)	4(4.71)	0(0.0)	76(89.41)	3(3.53)	6(7.06)	0(0.0)

significant difference in the genus level identification. For the identification performance results of sample from separate gel blood clotting tube we can found that, EXS 3000 correctly identification 78 (91.76%) strains in species level and 3 strains at genus level, Autof ms1000 correctly identification 76 (89.41%) strains at species level and 3 strains at genus level. The no reliable identification result of EXS3000 is 6 and 4 strains for blood culture kit samples and Separate gel blood clotting tube samples. The Autof ms1000 produced 7 and 6 cases with no reliable results for blood culture kit samples and Separate gel blood clotting tube samples respectively. There is no misidentification result for these two instruments.

Discussion

MALDI-TOF MS systems have been widely implemented in many clinical microbiology laboratories, providing tools for rapid, accurate, and cost-effective identification of cultured bacteria and fungi in clinical microbiology.^{9,10} This study aimed to evaluate the accuracy of MALDI-TOF MS to microorganism identification, choosing two instruments from different companies, and the performance of these instruments will be compared, thus extending the use of this technology to a novel application.

Through the comparison of test results show that, the species-level identification performance of the EXS3000 arrived at 91.39% and 90.91% for Autof ms1000. Conventional extraction methods were used in test samples to achieve these results, consistent with the methods used in clinical microbiology laboratories. It is obviously that similar identification results were obtained at species-level and genus-level (91.39% and 6.70% for EXS3000, 90.91% and 7.18% for Autof ms1000). The EXS3000 provided 4 misidentification results. Two of them from gram-negative bacteria and others from filamentous fungi. Autof ms1000 also provide 4 unreliable identification results. Both instruments have a similarity performance in the ability to correctly identify Enterobacteriaceae, identification results all arrived at species level. The exception is *Salmonella* and *Shigella*, for which serological identification is required. Compared with CPS methods identification of nonfermenting Gram-negative bacteria, MALDI-TOF MS identification is rapid and convenient.

Streptococcus pneumoniae has important clinical medical significance, it is a major human pathogen and must be reliably and accurately identified in the clinical microbiology Laboratory, our test results show that, both MALDI-TOF MS systems can identified it correctly, it is consistent with reported by others.¹¹⁻¹³ However, a part of *S. pneumoniae* isolates were misidentified. Similar misidentification, such as *S. mitis*, were misidentified as *S. pneumoniae*, has been reported by others.¹⁴⁻¹⁶ This misidentification can be explained by the reason of *S. pneumoniae* is similar to *S. mitis* cause only one strain of *S. pneumoniae* were used in this study, therefore we cannot ensure that all *S. pneumoniae* can be correctly detected by the MALDI-TOF MS system, but it has also proved that the technology has the ability to identify the *S. pneumoniae* to ensure the identification, supplementary tests such as optochin disk susceptibility and bile solubility can used for verification. Therefore, MALDI-TOF MS analysis cannot be used alone for identification of these organisms in all laboratories.^{17,18}

It has been reported that MALDI-TOF MS can accurately identify *Enterococci* at the species level¹⁹ the result of our study also verifies this conclusion, all *Enterococci* isolates were correctly identified to the species level by both systems. *Lautropia mirabilis* cannot be correctly identification by EXS3000, because *Lautropia mirabilis* is not included in the EXS3000 database.

Due to the differences in antimicrobial susceptibility test of filamentous fungi, the clinical selection of antibacterial drugs is different; therefore, rapid and accurate identification of filamentous fungi to the species level is essential to improve the antifungal efficacy. At present, most microbiology laboratories still use Traditional morphological methods to identify filamentous fungi, but it is difficult and time-consuming to identify strains with no typical characteristics, which is not

conducive to rapid and precise treatment.^{20,21} There have been reports on the application of MALDI-TOF MS technical to identify filamentous fungi. Twenty-seven strains of filamentous fungi were used in this study. The results show that EXS3000 and Autof ms1000 achieve 81.82% and 78.79% at species level respectively. Because filamentous fungi have great differences in different growth cycles, the fingerprint spectra detected in different periods may have differences. Usually we will collect spectra at different growth periods to create database. For EXS3000, the identification results show that five strains of *Cladosporium* were used for test, and four of them were identified at species level, only one strain result no reliable, because the pre-treatment of the strain may not be done properly, leading to unbelievable test results. From this test, it is proved that MALDI-TOF MS can be used in the identification of filamentous fungi.

Mycobacteria are mainly composed of *Mycobacterium tuberculosis* complex (MTC), *Mycobacterium leprae* and *nontuberculous mycobacteria* (NTM), and the main ones that can be cultivated clinically are MTC and NTM. NTM and MTC are both acid-fast bacilli. The clinical symptoms, pathological changes and imaging studies caused by pulmonary infection are very similar. It is easy to be misdiagnosed as *M. tuberculosis* infection or misdiagnosed as drug-resistant *M. tuberculosis* infection due to resistance to rifampin, resulting in diagnosis and treatment failed. Different NTM infection treatment drugs and drug resistance patterns are different, so accurate and rapid identification of bacterial species in the early stage of the disease is of great significance for clinical diagnosis and targeted treatment.^{22–24} Commonly used methods for identification of *mycobacterial* species in primary Hospitals are P-nitrobenzoic acid (PNB) and Thiophene-2-carboxyhydrazine (TCH) medium growth test methods, the *mycobacteria* can be initially identified as MTC and NTM, with low accuracy and time-consuming. MALDI-TOF MS technology developed in recent years. The characteristic peaks are obtained by detecting the protein composition of the sample and compared with the bacterial fingerprint in the database to identify the bacterial genus. Nineteen strains of *Mycobacterium* were used for test, the EXS3000 achieved species-level identification for 43 (75.44%) strains, the Autof ms1000 attain species-level identification for 39 (68.42%) strains. The results show that the MALDI-TOF MS technology can be used in the identification of *mycobacteria*, but the current instrument detection results need to be further optimized.

Bloodstream infections (BSIs) are related to high mortality and morbidity. Rapid administration of effective antimicrobial treatment is crucial for patient survival. MALDI-TOF MS technology can be used to identify pathogens directly from blood culture bottles speed up diagnosis of BSIs. The results show that, for blood culture kit sample the EXS3000 correctly identified 77 (90.59%) strains at the species level and 2 strains at the genus level, the Autof ms1000 correctly identified 74 (87.06%) strains at species level and 4 strains at the genus level. By comparison, we found that EXS 3000 is slightly better than Autof ms1000 in species-level identification, and there is no significant difference in the genus level identification.

In summary, the two instruments have similar identification performance, both easy to operate, fast for loading the target plate and achieving vacuum. They are both benchtops. However, cause the Autof ms1000 has an external vacuum pump, which makes more noise, they also have the same pre-sample extraction steps. Nevertheless, for the identification of gram-positive bacteria, filamentous fungi and mycobacteria at the species level, the identification result of EXS3000 is slightly better than that of Autof ms1000.

Conclusion

With the advancement of science and technology, more and more efficient and convenient detection methods are used in clinical detection, and the phenomenon of MALDI-TOF MS technology being used in a wider range of fields has been confirmed. In order to make this technology continue to develop and improve, we also need to continue to expand the strain fingerprint database and continue to improve the algorithm.

Ethical Approval and Consent to Participate

The study obtained approval from the ethics committee of Shanghai East Hospital, Tongji University School of Medicine (No.2018-099). The need for informed consents was waived by the Clinical Research Ethics Committee.

Funding

This work was supported by the National Natural Science Foundation [grant number 81971990], Key Discipline of Public Health in Shanghai [grant number GWV-10.1-XK04], Excellent Technology Leader in Shanghai [grant number 20XD1434500].

Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Yuan Y, Yao Z, Xiao E, et al. The first imported case of melioidosis in a patient in central China. *Emerg Microbes Infect.* 2019;8(1):1223–1228. doi:10.1080/22221751
2. Harch SAJ, Currie BJ, Papanicolas L, et al. Utility of a rapid lateral flow assay to resolve erroneous identification of *Burkholderia pseudomallei* as *Burkholderia thailandensis* by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. *J Clin Microbiol.* 2018;56(12). doi:10.1128/JCM.01437-18
3. Jang KS, Kim YH. Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. *J Microbiol.* 2018;56(4):209–216. doi:10.1007/s12275-018-7457-0
4. Tsuchida S, Umemura H, Nakayama T. Current status of matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) in clinical diagnostic microbiology. *Molecules.* 2020;25(20):4775. doi:10.3390/molecules25204775
5. Hou TY, Chiang-Ni C, Teng SH. Current status of MALDI-TOF mass spectrometry in clinical microbiology. *J Food Drug Anal.* 2019;27(2):404–414. doi:10.1016/j.jfda.2019.01.001
6. Angeletti S, Ciccozzi M. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry in clinical microbiology: an updating review. *Infect Genet Evol.* 2019;76:104063. doi:10.1016/j.meegid.2019.104063
7. Yingchun X, Fen Q, Jianzhong Z, et al. Expert consensus on clinical microbial mass spectrometry application in China. *Chin J Nosocomiol.* 2016;26(11):I–VIII.
8. CLSI. Interpretive criteria for identification of bacteria and fungi by targeted DNA sequencing. Clinical and Laboratory Standards Institute; 2018.
9. Ge MC, Kuo AJ, Liu KL, et al. Routine identification of microorganisms by matrix-assisted laser desorption ionization time-of-flight mass spectrometry: success rate, economic analysis, and clinical outcome. *J Microbiol Immunol Infect.* 2017;50(5):662–668. doi:10.1016/j.jmii.2016.06.002
10. Idelevich EA, Seifert H, Sundqvist M, et al. Microbiological diagnostics of bloodstream infections in Europe—an ESGBIES survey. *Clin Microbiol Infect.* 2019;25(11):1399–1407. doi: 10.1016/j.cmi.2019.03.024.
11. Yahiaoui RY, Goessens WH, Stobberingh EE, et al. Differentiation between *Streptococcus pneumoniae* and other viridans group streptococci by matrix-assisted laser desorption/ionization time of flight mass spectrometry. *Clin Microbiol Infect.* 2020;26(8):1088.e1–1088.e5. doi:10.1016/j.cmi.2019.11.024
12. Fan WT, Qin TT, Bi RR, et al. Performance of the matrix-assisted laser desorption ionization time-of-flight mass spectrometry system for rapid identification of streptococci: a review. *Eur J Clin Microbiol Infect Dis.* 2017;36(6):1005–1012. doi:10.1007/s10096-016-2879-2
13. Paramaiswari WT, Sidik NS, Khoeri MM, et al. Isolation and identification of optochin-resistant viridans group streptococci from the sputum samples of adult patients in Jakarta, Indonesia. *Int J Microbiol.* 2021;14:6646925. doi:10.1155/2021/6646925
14. Slotved HC, Facklam RR, Fuursted K. Assessment of a novel bile solubility test and MALDI-TOF for the differentiation of *Streptococcus pneumoniae* from other mitis group streptococci. *Sci Rep.* 2017;7(1):7167. doi:10.1038/s41598-017-07772-x
15. Harju I, Lange C, Kostrzewa M, et al. Improved differentiation of streptococcus pneumoniae and other *S. mitis* group streptococci by MALDI biotyper using an improved MALDI biotyper database content and a novel result interpretation algorithm. *J Clin Microbiol.* 2017;55(3):914–922. doi:10.1128/JCM.01990-16
16. Varghese R, Jayaraman R, Veeraraghavan B. Current challenges in the accurate identification of *Streptococcus pneumoniae* and its serogroups/serotypes in the vaccine era. *J Microbiol Methods.* 2017;141:48–54. doi:10.1016/j.mimet.2017.07.015
17. Nix ID, Idelevich EA, Schlattmann A, et al. MALDI-TOF mass spectrometry-based optochin susceptibility testing for differentiation of *Streptococcus pneumoniae* from other *Streptococcus mitis* Group Streptococci. *Microorganisms.* 2021;9(10):2010. doi:10.3390/microorganisms9102010
18. Idelevich EA, Schlattmann A, Kostrzewa M, Becker K. Development of a novel MALDI-TOF MS-based bile solubility test for rapid discrimination of *Streptococcus pneumoniae*. *Int J Med Microbiol.* 2020;310(3):151413. doi:10.1016/j.ijmm
19. Garza-González E, Bocanegra-Ibarias P, Dinh A, et al. Species identification of Enterococcus spp: whole genome sequencing compared to three biochemical test-based systems and two Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) systems. *J Clin Lab Anal.* 2020;34(8):e23348. doi:10.1002/jcla.23348
20. Baumbach CM, Müller S, Reuschel M, et al. Identification of zoophilic dermatophytes using MALDI-TOF mass spectrometry. *Front Cell Infect Microbiol.* 2021;11:631681. doi:10.3389/fcimb.2021.631681
21. Hamal P, Vavrova A, Mrazek J, et al. Identification of filamentous fungi including dermatophytes using MALDI-TOF mass spectrometry. *Folia Microbiol.* 2022;67(1):55–61. doi:10.1007/s12223-021-00917-6

22. Alcaide F, Amlerová J, Bou G, et al.; European Study Group on Genomics and Molecular Diagnosis (ESGMD). How to: identify non-tuberculous Mycobacterium species using MALDI-TOF mass spectrometry. *Clin Microbiol Infect.* 2018;24(6):599–603. doi:10.1016/j.cmi.2017.11.012
23. Rindi L, Puglisi V, Franconi I, et al. Rapid and accurate identification of nontuberculous mycobacteria directly from positive primary MGIT cultures by MALDI-TOF MS. *Microorganisms.* 2022;10(7):1447. doi:10.3390/microorganisms10071447
24. Rodriguez-Temporal D, Perez-Risco D, Struzka EA, et al. Evaluation of two protein extraction protocols based on freezing and mechanical disruption for identifying nontuberculous mycobacteria by matrix-assisted laser desorption ionization-time of flight mass spectrometry from liquid and solid cultures. *J Clin Microbiol.* 2018;56(4):e01548. doi:10.1128/JCM.01548-17

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>