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ORIGINAL ARTICLE

Routine identification of microorganisms by matrix-assisted laser desorption ionization time-of-flight mass spectrometry: Success rate, economic analysis, and clinical outcome

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Abstract *Background:* Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been widely used in microbial identification. This study evaluated the performance of MALDI-TOF MS and investigated the economic and medical impact of MALDI-TOF MS implementation.
Methods: A total of 12,202 clinical isolates collected from April to September 2013 were identified using MALDI-TOF MS, and the success rates in identifying isolates were analyzed. The differences in the processing time, cost of consumables, weight of waste, and clinical impact between MALDI-TOF MS and biochemical reaction were compared.
Results: MALDI-TOF MS successfully identified 96% of 12,202 isolates, including 96.8% of 10,502 aerobes, 90.5% of 1481 anaerobes, 93.8% of 81 yeasts, and 90.6% of 138 nontuberculous mycobacteria at the genus level. By using MALDI-TOF MS, the processing time for aerobes decreased from 32.5 hours to 4.1 hours, and that for anaerobes decreased from 71.5 hours to 46 hours.

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For detection of aerobes and anaerobes, the cost of consumables was estimated to decrease by US$0.9 per isolate, thus saving US$94,500 in total annual isolation. Furthermore, the weight of waste decreased six-fold, resulting in a reduction of 350 kg/month or 4.2 tons/year. MALDI-TOF MS also increased the percentage of correct antibiotics treatment for *Escherichia coli* and *Klebsiella pneumonia* from 56.1% to 75% and shortened the initiation time of the correct antibiotic action from 3.3 hours to 2.5 hours.
Conclusions: MALDI-TOF MS is a rapid, reliable, economical, and environmentally friendly method for routine microbial identification and may contribute to early appropriate antibiotic treatment in clinical settings.

Introduction

The conventional methods for identifying microorganisms in clinical microbiology laboratories are based on biochemical methods and gene sequencing identification techniques. However, these procedures take considerable time, and the results may be difficult to interpret occasionally because of indistinct reactions or outdated databases.[1,2] Recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been effectively used as a rapid method for identifying a wide array of microbial species.[3,4] In MALDI-TOF MS analysis, abundant structural proteins such as ribosomal proteins are extracted from an intact bacterial colony. The ionizing laser vaporizes the abundant structural proteins of microorganisms, and unique mass spectra are generated, having mass-to-charge ratio (m/z) peaks with varying intensities. The mass spectra of test isolates are sequentially compared with those in a reference database for identification. Unknown organisms can be identified by matching the organism's spectrum to the most similar spectrum in the database. Depending on the MALDI-TOF MS score, the genus and species identification for an organism may be accurate.

MALDI-TOF MS can provide advantages for a universal procedure of microbial identification. Only a small amount of an organism, typically a fraction of a single colony from primary culture plates, is required for analysis. Comparatively, a larger inoculum and subculture is often required for conventional biochemical methods or other automated systems. The differences in procedure time and cost per isolate between MALDI-TOF MS and biochemical identification have been shown in previous studies.[1,5] However, cost assessment related to the subcultures required for the biochemical method, secondary biochemical testing such as coagulase for staphylococci, and the annual maintenance cost of MALDI-TOF MS should be considered when implementing MALDI-TOF MS in clinical settings. Furthermore, biohazard waste generated daily from microbial cultures and laboratory analysis may affect human health, waste management costs, and the environment. Because of its relative simplicity and speed, MALDI-TOF MS enables reducing the time spent on microbial identification. Rapid identification of microorganisms may contribute to the early treatment of patients by using an appropriate antimicrobial therapy, thereby improving patient outcomes, reducing the potential for microorganisms to develop antimicrobial resistance, and lowering mortality among bacteremic patients with sepsis.[6–8]

Although the use of MALDI-TOF MS for microbial identification has been well established,[9,10] its performance in identifying success rates and scores among different microbial species has yet to be extensively evaluated in clinical practice by using numerous clinical isolates. In addition, evidence of the impact of MALDI-TOF MS on costs, waste reduction, and the clinical outcomes of patients remains limited. In April 2013, the laboratory at Linkou Chang Gung Memorial Hospital switched from the conventional biochemical method to MALDI-TOF MS for microbial identification. Over a 6-month period, we evaluated the success rate of MALDI-TOF MS in identifying clinically relevant microorganisms, including aerobic and anaerobic bacteria, yeasts, and nontuberculous mycobacteria (NTM), at a 4000-bed tertiary teaching hospital (Linkou Chang Gung Memorial Hospital). In addition, we compared the processing time, cost of consumables, weight of waste, and clinical outcome of microbial identification between MALDI-TOF MS and biochemical methods.

Materials and methods

Microorganism isolates

A total of 12,202 clinical isolates, comprising aerobes (n = 10,502), anaerobes (n = 1481), yeasts (n = 81), and NTM (n = 138), were included in this study. The clinical isolates were obtained from fresh clinical specimens at Linkou Chang Gung Memorial Hospital in Taiwan from April to September 2013.

Sample preparation and MALDI-TOF MS analysis

The microorganism identifications and data analyses were performed using the Bruker LT microflex MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany). Sample preparation methods for MALDI-TOF MS analysis were performed as recommended by the manufacturer's protocol. A direct smear method with a 70% formic acid overlay was used for preparing aerobic and anaerobic bacteria samples, and ethanol–formic acid extraction and silica bead-based extraction methods were performed for

preparing yeast and NTM samples, respectively. Spectra were analyzed using the MALDI Biotyper automation control and Bruker Biotyper 3.0 system software (Bruker Daltonics). The MALDI-TOF mass spectrum database used comprised 4613 reference microorganism spectra (4274 bacteria, 332 eukaryotes, and 7 archaea). Scores $\geq$ 2.0 were considered reliable for identifying bacteria at the species level, and scores $\geq$ 1.7 and $<$ 2.0 were considered reliable for identifying those at the genus level. Scores $<$ 1.7 indicated ambiguous identification, and such identifications were confirmed according to the Clinical and Laboratory Standards Institute standard method. Failure to obtain signals by using mass spectrometry was designated *no peaks found*, and failure to match a spectrum to a spectrum in the database designated *no reliable identification*.

Time to identification

All positive results of aerobic or anaerobic bacteria identified using the biochemical method in December 2012 or using MALDI-TOF MS in July 2013 were calculated. Culture time was defined as the time from specimen collection to the occurrence of a colony size large enough for identification. Time to identification is defined as the time from colony formation to the time at which the final result reported to a physician is obtained.

Costs

The costs of the biochemical method may vary slightly, depending on the individual microbial species to be identified. Because aerobic and anaerobic bacteria were the most frequently isolated in clinical samples, we estimated the average costs of 52,500 isolates (47,200 aerobic and 5300 anaerobic isolates), including the costs for culture media, reagents, and biochemical testing, from July to December 2012. The average costs per isolate (US$1.6) for the biochemical method was calculated by dividing the total cost (US$84,000) by the total number of isolates (n = 52,500). The cost (US$0.7) of using MALDI-TOF MS for identification included the cost of the α-cyano-4-hydroxycinnamic acid matrix, ion detector, laser, and protein standard.

Weight of biohazard waste

The biohazard waste generated using the biochemical method and MALDI-TOF MS was weighed daily in December 2012 and July 2013, respectively, and the data were used to estimate the weight of monthly biohazard waste.

Clinical features and outcomes of patients

All hospitalized patients older than 18 years with bacterial cultures positive for *Escherichia coli* or *Klebsiella pneumoniae* from October to December 2012 were included and compared with patients whose bacteria samples were identified using MALDI-TOF MS in the same period in 2013. Patients with infections before hospital admission were excluded from the study. The numbers of days of hospitalization and mortality rates of all patients were collected and compared between the two patient groups. The time to antibiotics refers to the time from specimen collection to appropriate antibiotic administration, and antibiotic treatments that matched the drug susceptibility patterns of bacteria isolates were designated *correct antibiotic treatments*.

Results

MALDI-TOF MS identification rates for aerobic and anaerobic bacteria

Table 1 shows a ranked list of aerobic and anaerobic bacteria reported during one 6 months of 2013. Of the 10,502 aerobic bacteria, 96.8% (10,170/10,502) of isolates were identified at the genus level (2.0 $>$ score $\geq$ 1.7), and 88.7% (9316/10,502) of isolates were identified at the species level (score $\geq$ 2.0) by using MALDI-TOF MS. Among the top 10 aerobic bacteria accounting for 67.7% of all isolates, the estimated identification success rates achieved using this method were 91.2% to 100% at the genus level and 78.8% to 99.3% at the species level. By using MALDI-TOF MS, high rates of species identification were achieved for aerobic Gram-negative bacteria, such as *E. coli* (94.7%), *K. pneumonia* (92.1%), *Pseudomonas aeruginosa* (97.5%), and *Proteus mirabilis* (99.0%), and Gram-positive bacteria, such as *Enterococcus faecalis* (99.3%), *Staphylococcus aureus* (98.2%), and *Streptococcus agalactiae* (99.2%).

MALDI-TOF MS failed to identify 3.2% (332/10,502) of aerobic bacteria. Among these, the failure to identify 4.5% (n = 42) of *K. pneumonia* and 1.7% (n = 29) of *E. coli* was mainly due to the absence of peaks. In addition, the failure to identify 3.9% (n = 36) of Coag (−) *Staphylococcus*, 8.8% (n = 29) of *Viridans streptococcus*, and 3.7% (n = 22) of *Acinetobacter baumannii* was mainly attributed to a lack of reliable identification. No considerable difference was evident between the Gram-negative and Gram-positive bacteria in success rates (96.8% vs. 97.2%).

MALDI-TOF MS also successfully identified 90.5% (1341/1481) and 78.5% (1163/1481) of anaerobic bacteria at the genus and species levels, respectively. These rates are lower than those for aerobic bacteria at both levels. The eight most common anaerobic bacteria accounted for 56% of total isolates and were estimated in the range of 55.1% to 100% at the genus level and 51.4% to 93.3% at the species level. The highest success rates at both the species (93.3%) and genus levels (100%) were those for *Bacteroides thetaiotaomicron*. However, the lowest success rate at the species and genus levels was that for *Peptostreptococcus* spp. (51.4% for species and 55.1% for genus), followed by *Prevotella* spp. (73.6% and 81.7%), resulting in 44.9% (n = 48) of *Peptostreptococcus* spp. and 18.3% (n = 36) of *Prevotella* spp. remaining unidentified. Identification failure was attributed primarily to the absence of reliable identification profiles. The overall failure rate in anaerobic bacteria identification was 9.5% (n = 140).

MALDI-TOF MS identification rates in yeasts and NTM

As shown in Table 2, the total success rates of species and genus identification for yeast by using MALDI-TOF MS were 77.8% (63/81) and 93.8% (76/81), respectively. Notably, the

Table 1 Routine identification performance of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for aerobic and anaerobic bacteria.

Microorganism	Total no.	No. (%) of isolates identified to		No. (%) of unidentified isolates		
		Species level	Genus level	Total	No peaks found	No reliable identification
Aerobes	10502	9316 (88.7)	10170 (96.8)	332 (3.2)	143 (1.4)	189 (1.8)
Gram-negative	6861	6130 (89.3)	6632 (96.7)	229 (3.3)	116 (1.7)	113 (1.6)
Escherichia coli	1671	1582 (94.7)	1642 (98.3)	29 (1.7)	25 (1.5)	4 (0.2)
Klebsiella pneumoniae	931	857 (92.1)	889 (95.5)	42 (4.5)	41 (4.4)	1 (0.1)
Pseudomonas aeruginosa	757	738 (97.5)	747 (98.7)	10 (1.3)	4 (0.5)	6 (0.8)
Acinetobacter baumannii	590	516 (87.5)	568 (96.3)	22 (3.7)	5 (0.8)	17 (2.9)
Proteus mirabilis	310	307 (99.0)	310 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others	2602	2130 (81.9)	2476 (95.2)	126 (4.8)	41 (1.5)	85 (3.3)
Gram-positive	3641	3186 (87.5)	3538 (97.2)	103 (2.8)	27 (0.7)	76 (2.1)
Coag (−) *Staphylococcus*	935	749 (80.1)	899 (96.1)	36 (3.9)	6 (0.6)	30 (3.2)
Staphylococcus aureus	613	602 (98.2)	611 (99.7)	2 (0.3)	0 (0.0)	2 (0.3)
Enterococcus faecalis	579	575 (99.3)	579 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Streptococcus agalactiae	389	386 (99.2)	388 (99.7)	1 (0.3)	1 (0.3)	0 (0.0)
Viridans streptococcus	330	260 (78.8)	301 (91.2)	29 (8.8)	9 (2.7)	20 (6.1)
Others	795	614 (77.2)	760 (95.6)	35 (4.4)	11 (1.4)	24 (3.0)
Anaerobes	1481	1163 (78.5)	1341 (90.5)	140 (9.5)	43 (2.9)	97 (6.5)
Gram-negative	903	751 (83.2)	845 (93.6)	58 (6.4)	18 (2.0)	40 (4.4)
Bacteriodes fragilis	215	200 (93.0)	213 (99.1)	2 (0.9)	0 (0.0)	2 (0.9)
Prevotella spp.	197	145 (73.6)	161 (81.7)	36 (18.3)	11 (5.6)	25 (12.7)
Veillonella spp.	63	52 (82.5)	59 (93.7)	4 (6.3)	0 (0.0)	4 (6.3)
Bacteroides thetaiotaomicron	60	56 (93.3)	60 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others	368	298 (81.0)	352 (95.7)	16(4.3)	7 (1.9)	9 (2.4)
Gram-positive	578	412 (71.3)	496 (85.8)	82 (14.2)	25 (4.3)	57 (9.9)
Peptostreptococcus spp.	107	55 (51.4)	59 (55.1)	48 (44.9)	13 (12.1)	35 (32.7)
Propionibacterium acnes	84	65 (77.4)	76 (90.5)	8 (9.5)	6 (7.1)	2 (2.4)
Peptostreptococcus micros	62	56 (90.3)	62 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Peptostreptococcus anaerobius	42	37 (88.1)	38 (90.5)	4 (9.5)	1 (2.4)	3 (7.1)
Others	283	199 (70.3)	261 (92.2)	22 (7.8)	5 (1.8)	17 (6.0)

success rates for *Candida glabrata* reached 100% at both the species and genus levels. However, the genus identification rate for *Candida parapsilosis* was only 85.7%, and no peaks were determined in the corresponding MALDI-TOF MS results of two unidentified isolates. These results showed that the success rates of yeast identification were highly dependent on the yeast species.

MALDI-TOF MS was used to analyze 138 NTM isolates. The success rates for the rapid- and slow-growing types were slightly different (Table 2). The slow-growing types had a higher genus identification rate (92.2%) than that of the rapid-growing types (87.5%), but their species identification rate (68.8%) was lower than that of the rapid-growing types (87.5%). The MALDI-TOF MS analysis of unidentified isolates indicated that no peaks were found in the slow-growing type, and failure to identify isolates of the rapid-growing type was mainly due to a lack of reliable identification.

Comparisons of time to identification, cost, and biohazard weight between biochemical and MALDI-TOF MS identification

To ascertain the differences in the time of microbial identification, the processing times for aerobic and anaerobic bacteria identification were evaluated first (Table 3). The culture time required for bacterial colony formation (from sample receipt to colony formation) was similar for the two methods. Time to identification (from colony formation to complete bacterial identification) was markedly lower for MALDI-TOF MS than for biochemical identification. Specifically, the time to identification decreased from 32.5 hours to 4.1 hours (7.93-fold) for aerobes and from 71.5 hours to 46 hours (1.55-fold) for anaerobes. Furthermore, the cost and waste of bacterial identification by using these two methods was compared. MALDI-TOF MS identification reduced the cost from US$1.6 to US$0.7 per isolate (2.29-fold) and reduced the weight of biohazard waste from 420 kg/month to 70 kg/month (6-fold).

Impact of MALDI-TOF MS identification on clinical outcomes

E. coli and *K. pneumoniae* are two commonly isolated Gram-negative bacteria of clinical relevance in Taiwan. To investigate the effects of using MALDI-TOF MS for rapid bacterial identification on antimicrobial therapies and clinical outcomes, patients positive for *E. coli* or *K. pneumonia* identified using biochemical testing ($n = 114$) or MALDI-TOF MS ($n = 148$) were compared over identical

Table 2 Routine identification performance of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for yeasts and nontuberculous mycobacteria (NTM).

Microorganism	Total no.	No. (%) of isolates identified to		No. (%) of unidentified isolates		
		Species level	Genus level	Total	No peaks found	No reliable identification
Yeast	81	63 (77.8)	76 (93.8)	5 (6.2)	4 (4.9)	1 (1.2)
Candida glabrata	19	19 (100.0)	19 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida tropicalis	16	12 (75.0)	15 (93.7)	1 (6.3)	1 (6.3)	0 (0.0)
Candida albicans	15	11 (73.3)	15 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Candida parapsilosis	14	9 (64.3)	12 (85.7)	2 (14.3)	2 (14.3)	0 (0.0)
Others	17	12 (70.6)	15 (88.2)	2 (11.8)	1 (5.9)	1 (5.9)
NTM	138	104 (75.4)	125 (90.6)	13 (9.4)	6 (4.3)	7 (5.0)
Slow-growth	90	62 (68.8)	83 (92.2)	7 (7.8)	5 (5.5)	2 (2.2)
Mycobacterium chimaera intracellulare group	30	22 (73.3)	27 (90.0)	3 (10.0)	2 (6.6)	1 (3.3)
Mycobacterium gordonae	13	9 (69.2)	13 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mycobacterium kansasii	9	7 (77.8)	9 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mycobacterium avium	8	5 (62.5)	7 (87.5)	1 (12.5)	1 (12.5)	0 (0.0)
Mycobacterium porcinum	6	6 (100.0)	6 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mycobacterium intracellulare	5	3 (60)	4 (80.0)	1 (20.0)	1 (0.2)	0 (0.0)
Others	19	10 (52.6)	17 (89.5)	2 (10.5)	1 (5.3)	1 (5.3)
Rapid-growth	48	42 (87.5)	42 (87.5)	6 (12.5)	1 (2.0)	5 (10.4)
Mycobacterium fortuitum	28	26 (92.9)	26 (92.9)	2 (7.0)	0 (0.0)	2 (7.1)
Mycobacterium abscessus	14	12 (85.7)	12 (85.7)	2 (14.3)	0 (0.0)	2 (14.3)
Others	6	4 (66.7)	4 (66.7)	2 (33.3)	1 (16.7)	1 (16.7)

Table 3 Comparison of processing time, cost, and waste weight between matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and biochemical identification.

Method	Culture time (h)		Time-to-identification (h)		Average cost per identification (US$)	Weight of biohazard waste per month (kg)
	Aerobes	Anaerobes	Aerobes	Anaerobes		
Biochemical identification	24.9	49.3	32.5	71.5	1.6	420
MALDI-TOF MS identification	23.3	45.6	4.1	46	0.7	70
Fold-reduction	1.07	1.08	7.93	1.55	2.29	6

3-month periods before and after MALDI-TOF MS implementation (Table 4). No statistically significant difference was observed between biochemical and MALDI-TOF MS identification regarding the mean age (64.9 $\pm$ 17.3 vs. 66.7 $\pm$ 14.5), male-to-female ratio (41/73 vs. 51/97), and number of hospitalization days (16.4 vs. 17.6). Implementing MALDI-TOF MS shortened the time required for selecting appropriate antibiotics for treatment from 3.3 days to 2.5 days ($p < 0.05$), and the percentage of antibiotic prescriptions that matched the antibiotic susceptibility pattern increased from 56.1% to 75% ($p < 0.05$). Although the mortality rate decreased from 9.6% to 6.1%, this difference did not reach statistical significance.

Discussion

Rapidly and accurately identifying microorganisms is essential for guiding antimicrobial therapy and improving patient outcomes. Microbial identification is achieved within minutes by using MALDI-TOF MS, whereas it requires hours or days using biochemical methods. In this study, MALDI-TOF MS successfully identified 11,712 of 12,202 (96%) isolates from four types of microorganisms at the genus level (score $\geq$ 1.7), namely 10,170 aerobes, 1341 anaerobes, 76 yeasts, and 125 NTM, during a 6-month study period (Tables 1 and 2), demonstrating the use of MALDI-TOF MS for accurately identifying microorganisms as previously described.[10–14] Similar results were observed for aerobic Gram-negative and Gram-positive bacteria at the genus (96.7% vs. 97.2%) and species levels (89.3% vs. 87.5%), respectively. Our data also showed that 90.5% and 78.5% of anaerobic isolates were identified at the genus and species levels, respectively (Table 1), which were comparable with previous studies.[15] These data indicate that MOLDI-TOF MS is reliable in identifying aerobic and anaerobic bacteria, although the success rates are lower for the aerobes *V. streptococcus* and *K. pneumonia* and the anaerobes *Peptostreptococcus* spp. and *Prevotella* spp.

Table 4 Antibiotic treatment and patient outcomes for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and biochemical identification.

	Biochemical identification	MALDI-TOF MS identification
Patient		
No.	114	148
Age (y)	64.9 ± 17.3	66.7 ± 14.5
Male/female	41/73	51/97
Escherichia coli/ *Klebsiella pneumonia*	96/18	126/22
Outcome		
Hospitalization day	16.4 ± 9.2	17.6 ± 9.4
Correct antibiotic treatment	56.1%*	75%*
Time to antibiotics (d)	3.3 ± 1.6*	2.5 ± 0.9*
Mortality rate	9.6%	6.1%

*$p < 0.05$.

For identifying *Candida* species, we used an ethanol—formic acid extraction method, and identified < 80% of nonglabrata *Candida* species isolates (Table 2). However, previous studies showed that the identification rate significantly increased from 65.8% to 88% by using the direct smear method with formic acid overlay and reducing Biotyper threshold from ≥ 2.0 to ≥ 1.7.[16] Furthermore, De Carolis et al used an in-house library database, and 99.5% of 4232 yeast isolates were identified at the species level (with scores of ≥ 2.0).[17] For *Mycobacterium* identification, our results indicated that the Bruker system identified only 75.4% of 138 NTM isolates (Table 2). However, the Bruker system achieved species-level identification for 87.4% of NTM isolates after comparing with those data in an augmented database of 123 clinical *Mycobacterium* strains.[18] To achieve acceptable species-level identification, further research is required for investigating analytical factors for optimal yeast and *Mycobacterium* identification in clinical laboratories, including a fast sample preparation procedure, modification of the Biotyper threshold, and the development of in-house library databases.

The advantage of using MALDI-TOF MS for microbial identification pertains to cost. Our results showed that MALDI-TOF MS yielded a 2.29-fold reduction in cost. The cost saving per isolate reached US$0.9, and the total saving per year was approximately US$94,500 (according to 47,200 aerobic and 5300 anaerobic bacteria reported in the second half of 2012) when protein standards and instrument maintenance were considered (Table 3). In a recent report, Seng et al described an 11-year experience in the routine identification of clinical isolates, including 40 months of MALDI-TOF MS usage and 91 months of conventional phenotypic identification. MALDI-TOF MS identified 284,899 isolates of 459 species among 286,842 clonal isolates and reduced the cost five-fold compared with conventional phenotypic identification (Gram staining, API, Vitek 2 system identification), also showing that MALDI-TOF MS significantly reduced the cost of microbial identification.[19] Another benefit of MALDI-TOF MS identification is reduction of indirect costs such as that of waste management. Using MALDI-TOF MS can reduce waste by 350 kg/month or 4.2 tons/year; thus, it is more environmentally friendly than biochemical methods, which generate over 400 kg of biohazard waste/month (Table 3). A limitation of the current study is that the results are based on data collected for a short period, and bacteria activity is assumed to be constant for half a year. Nevertheless, our study demonstrated that implementing MALDI-TOF MS reduced the laboratory cost and the number of isolates requiring biochemical testing for identification.

In general, only a small fraction of a single colony or a small quantity of a bacterial suspension is required for MS identification. Therefore, an improved workflow was developed after implementing MALDI-TOF MS. The culture time for MS identification slightly decreased relative to that of the biochemical method because MS identification entails using a lower quantity of inoculants and requires no prolonged incubation (Table 3). Time to identification significantly decreased from 32.5 hours and 71.5 hours in the biochemical method to 4.1 hours and 46 hours in MALDI-TOF MS for aerobes and anaerobes, respectively (Table 3). Early identification increases the chances for successful treatment. Hence, early and empirical antibiotic treatment has been associated with a significant reduction in the mortality of bloodstream infection.[20] In this study, we evaluated the effects of using MALDI-TOF MS for *E. coli* and *K. pneumonia* identification on antimicrobial therapies and clinical outcomes. The prevalence of antimicrobial resistance in *E. coli* and *K. pneumonia* remains low, but has increased in recent years in Taiwan.[21] Through rapid bacterial identification by using MALDI-TOF MS, antibiograms representing the typical susceptibility of identified species can serve as a guide for empiric antimicrobial therapy. For *E. coli* and *K. pneumonia*, MALDI-TOF MS implementation facilitates beginning empirical antibiotic treatment earlier (3.3 vs. 2.5 days) and increases the proportion (56.1% vs. 75%) of prescriptions matching drug-susceptibility patterns (Table 4). A significant reduction was not observed in the hospitalization days, although previous studies have shown that the mean hospital stay decreased after MALDI-TOF MS identification and rapid antimicrobial susceptibility testing were implemented.[22] Besides, MALDI-TOF MS combined with antimicrobial stewardship team (AST) intervention has demonstrated a significant reduction of mortality from 20.3% to 14.5% on bloodstream infection.[23] Our data also showed a reduced mortality rate (9.6% to 6.1%) after MALDI-TOF MS implementation, but our sample size was insufficient to detect statistical significance.

This study provides insight into the economic benefits of MALDI-TOF MS and demonstrated its performance in identifying numerous clinical isolates, reflecting its potential to become a major identification system in teaching hospitals. Expanding the database by increasing numbers of reference strains will contribute to the overall reliability of identification. Moreover, developing a suitable protocol for some bacteria to lyse cell walls efficiently may facilitate obtaining high-quality spectra in the future. MALDI-TOF MS enables rapid and reliable identification of microorganisms and assigns a microbial cause to an infection, thus facilitating the provision of effective antimicrobial treatments to improve the clinical outcome.

Conflicts of interest

The authors declare no conflict of interest.

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