Comparison of bacterial identification by MALDI-TOF mass spectrometry and conventional diagnostic microbiology methods: agreement, speed and cost implications

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ABSTRACT

Identification of microbial pathogens still relies primarily on culture and phenotypic methods, which is labour-intensive and time-consuming. In this study, identification of bacteria with valid standard identification using BD Phoenix, API panels and other recommended procedures is compared to identification with matrix-assisted laser desorption/ionisation–time of flight (MALDI-TOF) mass spectrometry using the MALDI Biotyper (Bruker Daltonics) in the setting of a routine NHS diagnostic microbiology laboratory. In total, 928 bacterial isolates obtained from blood (n=463), wounds and pus (n=208), respiratory tract (n=100), faeces (n=86) and urines (n=71) were analysed. There were 721 (77.7%) isolates with a MALDI Biotyper score ≥2.0, indicating secure genus and probable species identification; and 149 (16.1%) isolates with a score ≥1.7 and <2.0 indicating probable genus identification. The isolates with scores of ≥2.0 and ≥1.7 comprised 31 and 33 genera and 65 and 67 species, respectively. Overall, 99.4% and 99.1% of organism identifications were in agreement between the MALDI Biotyper and conventional identification at the genus level, and 89.3% and 87.8% at species level when analysing organisms with MALDI Biotyper scores ≥2.0 and ≥1.7, respectively. With many but not all organisms, identification at the genus level is sufficient; however, MALDI Biotyper separation of 208 staphylococci into *Staphylococcus aureus* and coagulase-negative staphylococci was always correct when scores were ≥1.7. First results were obtained after 5–10 min and analysis of a full 96-well target plate was completed in approximately 90 min. Substantial savings of between £1.79 and £2.56 per isolate, depending on the cost model of acquisition of the MALDI Biotyper system and number of isolates tested, would be realised when all 928 isolates were identified using the MALDI Biotyper and disk-susceptibility testing when compared to the cost for 618 Phoenix ID panels and 158 API panels and disk-susceptibility tests only (i.e., not taking into account costs incurred for identification of the remaining 152 mixed isolates). Microbial identification by MALDI Biotyper offers a rare opportunity for significant cost-neutral or even cost-saving quality improvements in medical diagnostics.

KEY WORDS: Microbiology.
Spectrometry, mass, matrix-assisted laser desorption-ionization.

Introduction

Clinical microbiology laboratories are under increasing pressure to deliver a service that is both reliable and cost-effective, with a rapid turnaround time. However, pathogen identification and antimicrobial susceptibility testing still relies primarily on culture and phenotypic analysis, which is time-consuming and labour-intensive, requiring 24–36 h from initial pathogen isolation to result. Semi-automated identification and susceptibility testing systems such as Phoenix (Becton-Dickinson), Vitek 2 (bioMérieux) and Microscan Walkaway (Siemens) have overcome some of this delay, but they are still time-consuming.[1]

Polymerase chain reaction (PCR)-based microbial identification methods have been introduced, which are ideally suited to improve turnaround times of microbial identification; however, their routine uptake in diagnostic microbiology laboratories has been hampered by the fact that these methods are not universal and only address certain subsets of organisms unless using 16S rRNA gene sequencing.[2] The real-time PCR approach has entered clinical microbiology laboratories for selected applications; for example, for speciation and susceptibility testing of staphylococci even directly from clinical specimens or positive blood cultures, and for rapid detection of pathogens in cerebrospinal fluid (CSF).[3–7]

Matrix-assisted laser desorption/ionisation–time of flight mass spectrometry (MALDI-TOF MS) is another molecular approach in diagnostic microbiology.[8,9] The MALDI-TOF MS

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technique uses soft laser ionisation on intact bacteria or bacterial extracts, detecting the peptide and protein ions, predominantly universally present ribosomal proteins,[10] according to their relative masses and charges, thus generating species-specific spectra based on their mass/charge ratio (m/z).[8] Over the past few years, different bacterial spectra databases have been developed, using various algorithms to detect species-specific conserved peaks, which are then used as biomarkers to identify the bacterial species.[8,11,12]

Such work has led to the development of the Biotyper database (Bruker Daltonics, Bremen, Germany) containing over 3000 well-characterised entries, which has been evaluated for identification of medically important bacterial genera and yeasts,[11,13–26] and has been recently evaluated for microbial identification in different diagnostic clinical microbiology laboratory settings.[27–32] An alternative system uses the Saramis database (AgnosTec, Potsdam, Germany), usually together with Axima Assurance (Shimadzu Corporation) MALDI-TOF MS equipment, now marketed as Vitek MS (bioMérieux).[32,33]

The aim of this study is to assess agreement of MALDI-TOF MS and MALDI Biotyper microbial identification with the currently used, commercially available automated and manual phenotypic identification methods in a busy routine NHS diagnostic microbiology laboratory, as well as to evaluate cost implications of introduction of this new technology in clinical microbiology testing.

Materials and methods

Setting and bacterial isolates

The Public Health Wales Microbiology ABM Swansea laboratory serves a population of approximately 350,000, including three major acute hospitals of Abertawe-Bro Morgannwg University Health Board (approximately 1570 beds) with multiple secondary and tertiary services including haematology with a regional bone-marrow transplant centre, neonatal ICU subregional centre, cardiac surgery, and the Welsh Centre for Burns and Plastic Surgery. Bacterial isolates were recovered from clinical specimens cultured using Health Protection Agency (HPA) standard operating procedures (www.hpa-standardmethods.org.uk/pdf_sops.asp).

Over a period of six months in 2009, organisms of clinical significance that were judged to require identification by conventional phenotypic methods were also subjected to identification with MALDI-TOF MS using the MALDI Biotyper (Bruker Daltonik, Bremen, Germany). Isolates with secure genus identification, probable species identification or better with the MALDI Biotyper (MALDI Biotyper score ≥2.0; see below) and a valid reference identification using established phenotypic methods were evaluated. Additionally, all isolates with probable genus identification or better by MALDI Biotyper (MALDI Biotyper score ≥1.7) and valid reference identification were analysed separately. No attempt was made to resolve discrepant identification by 16S rDNA sequencing, which was not routinely available in the authors' laboratory. The 16S rDNA sequence is seen as the current gold standard for identification; however, a number of discrepancies could not be resolved using this approach in a previous study.[27]

The nature of isolates reflects the usual identification strategy in many NHS laboratories, where currently many isolates from urines or respiratory specimens remain non-speciated. Isolates were obtained from blood (n=463), wounds and pus (n=208), respiratory tract (n=100), faeces (n=86) and urines (n=71).

MALDI-TOF mass spectrometry

Steel MALDI-TOF target plates (96-spot) were spotted using a disposable inoculation loop with a very thin film of material from a bacterial colony in duplicate and covered with 1-μL matrix solution, a saturated solution of alpha-cyano-4-hydroxy cinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid (all LC-MS quality; Sigma, Gillingham, UK). The plate was allowed to air dry and was analysed in a Bruker Daltonic Microflex LT benchtop MALDI mass spectrometer containing a 60 Hz nitrogen laser and microSCOUT MALDI ion source with extended pulsed ion extraction PAN mass range focusing. The spectra formed were analysed with MALDI Biotyper 2.0 software (Bruker Daltonics), which matched the generated spectra with reference spectra of more than 3000 microorganisms. The manufacturer recommends evaluation criteria for the MALDI Biotyper to determine accuracy of species identification: scores ≥2.300–3.000 indicating highly probable species identification; scores ≥2.000–2.299 indicating secure genus identification, probable species identification; scores ≥1.700–1.999 indicating probable genus identification; and scores <1.700 meaning no reliable identification.

Phenotypic identification

Aerobic Gram-negative bacilli, staphylococci and enterococci were identified by BD Phoenix (Becton-Dickinson). The BD Phoenix panels used on 618 isolates were NMIC/ID-65 (n=171 isolates) and NMIC/ID-76 (n=170 isolates) for aerobic Gram-negative bacilli, PMIC/ID-63 (n=121 isolates) and PMIC/ID-67 (n=137 isolates) for Gram-positive bacteria, and SMIC/ID-9 (n=19 isolates) for streptococci. Confidence scores >90% were accepted to identify isolates to species level. A total of 158 isolates were identified by API (bioMérieux): API 20 E, API 20 NE, API 32 A and API NH.

In addition to a BD Phoenix panel, all staphylococci were tested for coagulase and clumping factor using Staph Xtra Latex (Pro-Lab Diagnostics), and a DNase plate was also tested. Among the streptococci (n=40 isolates), Lancefield antigens of β-haemolytic streptococci were determined using the Prolex latex agglutination system (Pro-Lab Diagnostics).

Streptococcus pneumoniae were identified by optochin susceptibility, colonial morphology and bile solubility. Anaerobes (n=51 isolates) were tested with API 32 A and confirmed at the Anaerobe Reference Laboratory, Public Health Wales Microbiology Cardiff, UK.

Campylobacter spp. (n=18 isolates) were identified only to genus level according to UK national guidelines. *Haemophilus* spp. (n=37 isolates) were identified by colonial morphology and requirement for X and V factors. *Moraxella catarrhalis* (n=6 isolates) were identified by colonial morphology and hydrolysis of tributyrin.

Cost comparison

Minimum cost of phenotypic testing, including staff costs (biomedical scientist midpoint band 6) is £3.86 for each API

Table 1. Agreement of MALDI Biotyper identification with conventional comparator identification.

	MALDI Biotyper score ≥2.0			MALDI Biotyper score ≥1.7		
Taxon	No. tested	Agreement at genus level	Agreement at species level	No. tested	Agreement at genus level	Agreement at species level
Enterobacteriaceae	296	292 (98.6%)	274 (92.5%)	313	308 (98.4%)	287 (91.6%)
Citrobacter spp.	9	9 (100%)	8 (88.9%)	9	9 (100%)	8 (88.9%)
C. amalonaticus	1	1	1	1	1	1
C. braakii	3	3	2	3	3	2
C. freundii	1	1	1	1	1	1
C. koseri	3	3	3	3	3	3
C. youngae	1	1	1	1	1	1
Escherichia coli	135	135 (100%)	135 (100%)	141	141 (100%)	141 (100%)
Enterobacter spp.	38	36 (94.7%)	20 (52.6%)	44	41 (93.2)	21 (47.7)
E. aerogenes	6	6	6	7	7	7
E. cloacae	16	16	14	16	16	14
E. asburiae	2	2	0	4	4	0
E. kobei	13	13	0	16	15	0
Klebsiella spp.	40	38 (95%)	37 (92.5%)	40	38 (95%)	37 (92.5%)
K. oxytoca	8	8	8	8	8	8
K. pneumoniae	32	30	29	32	30	29
Morganella morganii	6	6 (100%)	6 (100%)	6	6 (100%)	6 (100%)
Proteus spp.	15	15 (100%)	15 (100%)	15	15 (100%)	15 (100%)
P. mirabilis	13	13	13	13	13	13
P. vulgaris	2	2	2	2	2	2
Serratia spp.	38	38 (100%)	38 (100%)	42	42 (100%)	42 (100%)
S. marcescens	37	37	37	41	41	41
S. liquefaciens	1	1	1	1	1	1
Salmonella enterica	13	13 (100%)	13 (100%)	13	13 (100%)	13 (100%)
Buttiauxella gavinae	2	2 (100%)	2 (100%)	2	2 (100%)	2 (100%)
Pantoea agglomerans	0	0	0	1	1	1
Non-fermenters	98	98 (95.9%)	80 (81.6%)	122	119 (97.5%)	97 (79.5%)
Acinetobacter spp.	26	26 (100%)	13 (50.0%)	36	35 (97.2%)	19 (52.7%)
A. baumannii	6	6	5	6	6	5
A. lwoffii	3	3	3	3	3	3
A. ursingii	8	8	0	9	8	0
A. junii	2	2	0	5	5	0
A. grimontii	1	1	0	1	1	0
A. parvus	1	1	0	1	1	0
A. species	5	5	5	11	11	11
Brevundimonas diminuta	1	1	1	1	1	1
Pseudomonas spp.	45	45 (100%)	42 (93.3%)	45	45 (100%)	42 (93.3%)
P. aeruginosa	41	41	41	41	41	41
P. putida	1	1	0	1	1	0
P. geniculata	1	1	0	1	1	0
P. montelli	1	1	0	1	1	0
P. oryzihabitans	1	1	1	1	1	1
Elizabethkingia miricola	1	1	0	1	1	0
Burkholderia sp.	1	1	0	2	1	0
Stenotrophomonas maltophilia	24	24 (100%)	24 (100%)	35	35 (100%)	35 (100%)
Achromobacter sp.	0	0	0	2	1	0

Table 1 (continued). Agreement of MALDI Biotyper identification with conventional comparator identification.

	MALDI Biotyper score ≥2.0			MALDI Biotyper score ≥1.7		
Taxon	No. tested	Agreement at genus level	Agreement at species level	No. tested	Agreement at genus level	Agreement at species level
Gram-positive bacteria	247	247 (100%)	214 (86.6%)	347	347 (100%)	296 (85.3%)
Staphylococcus spp.	163	163 (100%)	144 (88.3%)	208	208 (100%)	182 (87.5%)
S. aureus	85	85	85	101	101	101
CoNS	78	78	78	107	107	107
S. epidermidis	55	55	43	75	75	58
S. hominis	7	7	5	8	8	5
S. warneri	2	2	0	2	2	0
S. haemolyticus	6	6	5	10	10	9
S. lugdunensis	1	1	1	2	2	2
S. capitis	7	7	5	8	8	6
Micrococcus	1	1	1	2	2	2
Enterococcus spp.	32	32 (100%)	26 (81.3%)	45	45 (100%)	37 (82.2%)
E. faecalis	15	15	11	25	25	20
E. faecium	17	17	15	20	20	17
Streptococcus spp.	21	21 (100%)	14 (66.7%)	40	40 (100%)	31 (77.5%)
S. agalactiae	3	3	3	5	5	5
S. anginosus	1	1	0	4	4	2
S. dysgalactiae	4	4	2	5	5	3
S. pyogenes	3	3	3	4	4	4
S. pneumoniae	10	10	6	21	21	10
Listeria monocytogenes	4	4	4	7	7	7
Lactobacillus sp.	1	1	1	1	1	1
Clostridium spp.	25	25 (100%)	25 (100%)	44	44 (100%)	44 (100%)
C. perfringens	9	9	9	10	10	10
C. difficile	14	14	14	30	30	30
C. paraputrificum	1	1	1	1	1	1
C. tertium	1	1	1	1	1	1
C. septicum	0	0	0	2	2	2
Gram-negative bacteria	80	80 (100%)	61 (96.8%)	88	88 (100%)	68 (97.1%*)
Campylobacter spp.	17	17	n.a.	18	18	n.a.
Haemophilus spp.	34	34	34	37	37	37
H. influenzae	33	33	33	34	34	34
H. parainfluenzae	1	1	1	3	3	3
Bacteroides fragilis	5	5	5	5	5	5
Moraxella catarrhalis	6	6	6	6	6	6
Neisseria spp.	11	11	11	12	12	12
N. gonorrhoeae	6	6	6	7	7	7
N. meningitides	4	4	4	4	4	4
N. flavescens	1	1	1	1	1	1
Pasteurella multocida	3	3	2	4	4	3
Plesiomonas shigelloides	1	1	1	2	2	2
Aeromonas culicicola	1	1	0	1	1	0
Aeromonas hydrophila	1	1	1	1	1	1
Fusobacterium necrophorum	1	1	1	2	2	2

*The 18 *Campylobacter* spp. were not included in the denominator as these bacteria are not routinely identified to the species level according to national guidelines.

and disk-susceptibility test; and £5.34 for a BD Phoenix panel (identification and susceptibility). The laboratory's minimum cost for disk-susceptibility testing, including staff costs, is approximately £1.14. Staph latex agglutination plus DNase tests cost a minimum of £0.47 per isolate.

Identification of isolates by the MALDI Biotyper (Bruker Daltonics Microflex LT mass spectrometer and MALDI Biotyper 2.0 software) were cost-estimated according to two options for acquiring the MALDI Biotyper system (i.e., a lease model over five years, and purchase of the instrument including a maintenance contract over five years). Costs per identification were then calculated for use of the instrument at maximum daily capacity; at 25% of maximum capacity; for the current number of identifications performed in the laboratory; and for the possible number of identifications for which the instrument could be used in the laboratory, to identify those organisms currently reported without identification (e.g., coliforms) (see Results and Table 2).

Results

The 928 isolates included in this study had valid identification with the conventional method used in the laboratory. There were 721 (77.7%) isolates with a MALDI Biotyper score ≥2.0; 149 (16.1%) isolates with a score of ≥1.7 and <2.0, and 58 (6.2%) isolates with a score of <1.7. The isolates with scores ≥2.0 and ≥1.7 were made up of 31 and 33 genera and 65 and 67 species, respectively. Overall, 99.4% and 99.1% of organism identifications were in agreement between the MALDI Biotyper and conventional identification at the genus level, and 89.3% and 87.8% at species level when analysing organisms with MALDI Biotyper scores ≥2.0 and ≥1.7, respectively.

Table 1 shows the breakdown of identifications and the concordance of results on a genus and species level for all identified isolates. In particular, Enterobacteriaceae with secure genus identification and probable species identification (MALDI Biotyper score ≥2.0) showed very high agreement at genus level (98.6%) and species level (92.5%).

The agreement in non-fermentative Gram-negative bacilli was 95.9% at genus level and 81.6% at species level. *Acinetobacter* spp. were the least concordant at the species level (50%) as *A. ursingii* and *A. parvus* were not included in the BD Phoenix database. When these were considered as *Acinetobacter* spp. the concordance improved to 85%. All *Pseudomonas aeruginosa* identifications agreed at species level with the two systems, and *Stenotrophomonas maltophilia* showed full concordance if four isolates identified as *P. hibiscicola* and one identified as *P. beteli* by MALDI Biotyper were also considered to be *S. maltophilia*, as which they were identified by BD Phoenix. This is reasonable as *P. hibiscicola* and *P. beteli* are old classifications for *S. maltophilia*.[27,28,30,34,35]

With Gram-positive bacteria, agreement was 100% at genus level and 86.6% at species level. While for many organisms identification to genus level may be acceptable, this is not the case with staphylococci, where a reliable distinction must be made at least between *Staphylococcus aureus* and the coagulase-negative staphylococci. Agreement in the identification and distinction between *S. aureus* and coagulase-negative staphylococci was 100%. Identification of enterococci was also in good agreement, although BD Phoenix identified a few strains as *E. casseliflavus/gallinarum*, which rarely occur in clinical specimens and stand out through low-level vancomycin resistance mediated by *vanC*.[36]

Table 2. MALDI Biotyper identification cost estimates.

Use of MALDI Biotyper	Cost per isolate (£)	
	Lease model	Purchase and service contract
25% (at 25% of full daily capacity)	2.30	1.50
40% (use for current identifications in laboratory)	1.28	0.82
64% (use for all possible identifications in laboratory)	0.79	0.51
100% (at full daily capacity)	0.51	0.33

Of particular interest was the excellent agreement of identification of *Clostridium* spp. including *C. difficile* at species level. Identification of β-haemolytic streptococci was in good agreement; however, difficulties noted with *Streptococcus pneumoniae* identification in other studies were also encountered.[27–29]

Identification of miscellaneous 'other bacteria' including *Haemophilus, Neisseria, Listeria* and anaerobes were in full agreement between MALDI Biotyper and conventional phenotypic methods. Of particular interest was the excellent concordance in identification of *Campylobacter* spp.; MALDI Biotyper identified 16/17 isolates as *C. jejuni* and the remainder as *C. coli*, which is in agreement with epidemiological expectations.[37]

The inclusion of isolates with a MALDI Biotyper score of ≥1.7 and <2.0 did not significantly affect the overall results obtained using the recommended score of ≥2.0 (Table 1). This is of particular importance for 45 additional isolates of staphylococci with MALDI Biotyper scores between 1.7 and 2.0, which were all correctly assigned to either *Staphylococcus aureus* or coagulase-negative staphylococci, indicating that MALDI Biotyper identification is also highly reliable for staphylococci at these lower scores. Similarly, 16 additional *Clostridium difficile* isolates were correctly identified at species level with scores between 1.7 and 2.0.

Out of the 58 (6.3%) isolates that had a score below 1.7 and therefore could not be identified using the MALDI Biotyper, 33 showed no peaks on testing. These consisted mostly of mucoid enterobacteria, which might give sufficient spectra when retesting using the extraction method, as suggested by the manufacturer.

Speed of analysis and cost implications

Experience with MALDI Biotyper identification showed that for single organisms the time from plate to result can be as fast as 5–10 min, while analysis of a full 96-well target plate was finished in approximately 90 min. Therefore, within the routine working hours of a busy clinical laboratory, up to 350 identifications can be achieved per day, which is well above the required capacity of most diagnostic microbiology laboratories.

The cost for MALDI Biotyper identifications depends on the cost model, either lease or purchase with service

contract, and the number of isolates tested per year. Table 2 shows cost estimates for the two models, taking into account use of MALDI Biotyper at full daily capacity and at 25% of full daily capacity. In addition, Table 2 shows cost estimates for the current number of identifications performed in the authors' laboratory as well as all possible identifications, including all isolates currently reported (e.g., coliforms and *Pseudomonas* spp.) without further identification.

For the cost calculations stated below, the authors estimated the cost for MALDI Biotyper identification at £0.51 to £1.28 per isolate, which is the lowest and highest cost estimate according to the current or possible number of identifications in their laboratory (Table 2). This is a conservative cost estimate compared with other recent studies estimating identification costs by MALDI Biotyper per isolate at €1.43 (£1.25),[28] US$ 0.50 (£0.32),[32] US$ 0.41 (£0.26)[38] and AUS$ 0.45 (£0.30).[39]

The study was not designed to allow formal cost analysis; however, cost comparison on the basis outlined above may in the first place take into consideration the following three items. Assuming the cost for 618 BD Phoenix ID panels and 158 API panels plus cost of disk-susceptibility testing for identification of all 928 isolates only (i.e., not taking into consideration any costs incurred through analysis of the remaining 152 mixed isolates), savings between £1.79 and £2.56 per isolate would have been realised if all 928 isolates had been identified using the MALDI Biotyper and disk-susceptibility testing. Taking into account the cost of phenotypic testing of the 435 enterobacteria and non-fermentative Gram-negative bacilli in this study using 341 BD Phoenix panels and 93 API panels plus disk-susceptibility testing, savings between £2.59 and £3.36 per isolate would be realised using the MALDI Biotyper for identification and disk-susceptibility testing. Identification of 347 Gram-positive isolates using 277 BD Phoenix panels alone would have saved between £1.84 and £2.61 per isolate when using MALDI Biotyper identification and disk-susceptibility testing.

In addition, the minimum identification of staphylococci using Staph Latex and DNase agar plates could be replaced by full identification of staphylococci by the MALDI Biotyper at no added cost at the lower cost estimate for use of the MALDI Biotyper MALDI-TOF MS system in the authors' laboratory.

Discussion

Recently, the use of MALDI-TOF MS to identify organisms in routine clinical microbiology laboratories has been reported, and it shows promising results with good correlation when compared with conventional phenotypic methods.[27–32] These studies are not easy to compare as either the recommended score level of ≥2.0 was used for identification at species level or even lower scores were regarded as acceptable identifications with scores ≥1.9[28] or ≥1.7.[29,32,39] In some studies, bacterial isolates with low scores were retested using the extraction method recommended by the manufacturer,[29–31] while other studies used only direct application of microbial growth from colonies.[27,28,32] The extraction method tends to result in higher scores, thereby allowing sufficient identification of some bacteria with low scores using the direct method.

The present study prospectively compared MALDI Biotyper identification to the automated and manual phenotypic methods currently employed in a routine NHS diagnostic microbiology laboratory setting. The MALDI Biotyper results were evaluated using cut-off scores ≥2.0, as suggested by the manufacturer, and additionally using scores ≥1.7 as a cut-off for acceptable identification. Reducing the cut-off point for MALDI Biotyper identification to a score of 1.7 had little effect on the overall agreement as the system identified bacterial isolates to the species level. However, further studies would be required to confirm this finding.

The results showed that the MALDI Biotyper produced rapid results that closely correlated with currently used phenotypic methods (Table 1). With enterobacteria, the concordance with phenotypic methods to the genus and species level was excellent. All *Salmonella enterica* isolates were identified accurately, without differentiating to the serovar level.[40] One of the problem groups was *Enterobacter* species where concordance at the genus level was 95% and at the species level only 52%. This arose because 13 isolates identified by the MALDI Biotyper as *E. kobei* were called *E. cloacae* (n=9 isolates) and four others only *Enterobacter* spp. *E. kobei* is phenotypically similar to *E. cloacae*.[41] *E. asburiae* is also closely related to *E. cloacae*, as which it was identified by BD Phoenix.[42] In a recent comparison between MALDI Biotyper and phenotypic methods, the discrepancies presented by *Enterobacter* could not be resolved at the species level by 16S rRNA sequencing.[27,30]

Acinetobacter spp. presented some difficulties, as the MALDI Biotyper identified eight as *A. ursingii* and another as *A. parvus*, and neither species was included in the BD Phoenix database. However, species identification of *Acinetobacter* spp. with manual and semiautomated commercial identification systems currently used in diagnostic microbiology (e.g., API 20 NE, Vitek 2, BD Phoenix and MicroScan WalkAway systems) have been problematic.[43]

Identification of staphylococci was concordant between the systems in distinguishing between *S. aureus* and coagulase-negative staphylococci, even when including isolates with scores ≥1.7 (Table 1); however, there were some discrepancies in the identification of different coagulase-negative staphylococci (Table 1). Most of the discrepant coagulase-negative staphylococci gave low confidence values with BD Phoenix.

Identification of staphylococci using the MALDI Biotyper was excellent when compared to phenotypic[14,18] and molecular identification using *rpoB* sequencing[13] and the identification of species-specific genes.[15] In a recent study, comparison of MALDI-TOF MS-based identification of coagulase-negative staphylococci with BD Phoenix and Vitek 2 using *sodA* gene sequencing as a baseline molecular identification revealed that BD Phoenix identification was the least reliable method, with 23.1% misidentification results, while MALDI-TOF MS misidentified only 1.7% and Vitek 2 only 13.7% of isolates, respectively.[44] Also, the Saramis database allows accurate MALDI-TOF MS-based identification of staphylococci, compared to molecular and phenotypic identification.[33]

Only 60% of *Streptococcus pneumoniae* strains were identified accurately by the MALDI Biotyper and this is in line with previous experience, and is thought to be related to the fact that too few reference spectra are present in the database.[27,28,45]

Campylobacter spp. were identified accurately in the present study, as were the *Haemophilus*, *Moraxella* and *Neisseria* groups.[16,20,46,47] *Campylobacter* spp. were assigned to *C. jejuni* (16/17) and *C. coli* (1/17) by the MALDI Biotyper, reflecting the epidemiology in a recent study from Scotland (90.4% *C. jejuni*).[37]

The strict anaerobes tested showed complete concordance with conventional phenotypic methods, including *Clostridium* spp. Compared to identification by the National Anaerobic Reference Laboratory, all 30 *C. difficile* isolates were correctly identified to species level. The MALDI Biotyper showed promise for identification of other *Clostridium* spp., including those of clinical importance in a previous study.[17] The MALDI Biotyper reduced significantly the time taken to identify strict anaerobes and this could help to change the attitude of many busy clinical microbiology laboratories that do not fully identify their anaerobes because of the expense and length of time required.

In conclusion, MALDI-TOF MS has great potential to improve the turnaround time of microbiological diagnosis and to significantly cut costs. To exploit the performance of the MALDI Biotyper system fully it needs to be linked to the laboratory information management system in a manner that would allow reading and reporting of plates to be streamlined. In the NHS diagnostic microbiology environment, use of the MALDI Biotyper would save between £1.84 and £3.36 per isolate of enterobacteria, non-fermentative Gram-negative bacilli and Gram-positive bacteria identified. In other studies and healthcare environments MALDI Biotyper identification was estimated to decrease cost by 60–89% compared to using API or BD Phoenix identification panels.[28,32,38] This will bring about the capacity to identify the many clinically significant organisms that remain unidentified in most NHS microbiology laboratories, such as many Enterobacteriaceae from urines and respiratory specimens.[48] The additional capacity available would allow use of MALDI Biotyper identification to replace or change current clinical microbiology processes (e.g., Gram stain analysis or screening for some stool pathogens),[28,32,47] not to mention the recent developments of using MALDI-TOF identification directly on clinical specimens (e.g., positive blood cultures[9,45,49–53] or urines[54]). Microbial identification by MALDI Biotyper offers a rare opportunity for significant cost-neutral or even cost-saving quality improvements in medical diagnostics. □

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