Comparison of two matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry methods and API 20AN for identification of clinically relevant anaerobic bacteria

Wafaa Y. Jamal, May Shahin and Vincent O. Rotimi

Department of Microbiology, Faculty of Medicine, Kuwait University, Kuwait

Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is suitable for high-throughput and rapid microbial diagnosis at relatively low cost and can be considered an alternative to conventional biochemical and molecular identification systems in clinical microbiological laboratories, including anaerobe laboratories. Two commercially available MALDI-TOF MS systems, Bruker Microflex MS and bioMérieux VITEK MS, were evaluated for the identification of 274 isolates of clinically significant anaerobic bacteria recovered from routine cultures of clinical specimens in parallel with blinded comparison with conventional biochemical (API 20AN) or molecular methods. All were recovered cultures obtained from patients attending the Mubarak Al Kabir Hospital, Kuwait, during a 6 month period. Discrepant results after two attempts at direct colony testing had failed to provide acceptable MALDI-TOF identification were resolved by gold-standard 16S gene sequencing. The VITEK MS gave high-confidence identification of the 274 isolates, all of which were correctly identified. The Bruker Microflex MS system also gave high-confidence identification for 272 of the 274. After discrepancy testing, the Bruker MS results agreed with biochemical or molecular methods for 89.1 % of the isolates at species level and 10.2 % at genus level (0.72 % were misidentified). The level of agreement with the VITEK MS was 100 % at both species and genus level; no isolates were misidentified. Our data suggest that implementation of MALDI-TOF MS as a first step for identification will shorten the turnaround time and reduce the cost in the anaerobe laboratory.

INTRODUCTION

Anaerobes are important causes of several infections in the brain, lung, pelvis and abdomen. However, their isolation in culture and identification in the routine diagnostic microbiology laboratory is difficult and time consuming (Jousimies-Somer et al., 2002). Phenotypic and biochemical methods need time, and commitment for several days, and sometimes they do not distinguish closely related species or may give incorrect or inconclusive results, especially with uncommon or fastidious organisms. Molecular methods, e.g. PCR-based amplification methods and sequencing (Drancourt & Raoult, 2005; Song, 2005), are expensive, not practical for routine use, and need technical expertise. In addition, they are not available to many clinical laboratories.

Recent development of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has shown it to be a useful method for identification of different micro-organisms. Several studies have reported the advantage and performance of MALDI-TOF systems compared with commercially available systems (Fedorko et al., 2012; Nagy et al., 2009; Seng et al., 2009). There are several commercially available MALDI-TOF MS systems with software and databases for identification of micro-organisms isolated from clinical specimens, e.g. Bruker MS (Microflex; Bruker Daltoniks), VITEK MS (bioMérieux) and Shimadzu MS (AXIMA; Shimadzu Corporation). They are used to identify aerobic and anaerobic bacteria (Fedorko et al., 2012; Nagy et al., 2009, 2012; van Veen et al., 2010; Veloo et al., 2011b), mycobacteria (Saleeb et al., 2011), Nocardia (Verroken et al., 2010) and yeasts (van Veen et al., 2010) isolated on solid media from clinical specimens. MALDI-TOF MS has also been recently used for the identification of bacteria and yeasts directly from positive blood culture bottles (Ferroni et al., 2010; Stevenson et al., 2010). The MALDI-TOF MS system appears to be associated with rapid turnaround time, low sample volume requirements and modest reagent costs.

Abbreviation: MALDI-TOF MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry.
The present study was undertaken to determine the ability of two MALDI-TOF MS systems (Bruker Microflex MS and bioMérieux VITEK MS) to identify clinically significant anaerobic bacteria in comparison with conventional identification by API 20AN (bioMérieux).

**METHODS**

**Setting.** The evaluation of the Bruker Microflex MS and VITEK MS was done in the routine Clinical Microbiology Laboratory, Mubarak Al Kabir Hospital, Kuwait, over a period of 6 months, June-December 2011. Identification with API 20AN and 16S RNA sequencing was carried out in the Anaerobe Reference Laboratory, Mubarak Al Kabir Hospital. This hospital is a 500-bed tertiary hospital with nine satellite clinics.

**Bacterial isolates.** A total of 274 isolates were recovered from routine examination of clinical specimens submitted to the Mubarak Al Kabir Hospital during the study period. They were from various sources, primarily derived from pus, blood cultures, tissues, intra-abdominal samples and wounds. A total of five genera and 14 species were encountered. The isolates were cultured on Brucella agar (Oxoid) supplemented with 5% sheep blood, 5 μg haemin ml⁻¹ and 1 μg vitamin K1 ml⁻¹ and incubated at 37 °C in an anaerobic atmosphere of 80% H₂, 10% CO₂ using the Anoxomat Anaerobic System (AN2CTS model; Mart Microbiology) for 48 h prior to the procedure. Isolates were processed within 2 h after removal from the incubator. One dedicated laboratory technologist operated each system.

**Routine identification.** Initial bacterial identification in the laboratory was carried out using the API 20AN system (bioMérieux) according to the manufacturer’s instructions. Each batch of Gram-negative isolates was run in parallel with control strains of *Bacteroides fragilis* ATCC 25285, and Gram-positive isolates with *Clostridium perfringens* type A strain ATCC 13124 and *Clostridium difficile* ATCC 70057.

**Measurements with the Bruker Microflex MALDI-TOF MS.** All isolates were tested in duplicate. The system was operated as described by Cherkaoui *et al.* (2010). The colonies were picked up from the anaerobic Brucella blood agar and inoculated onto the MALDI target plate. This system (comprising a Microflex MALDI-TOF mass spectrometer with flexControl software and the MALDI BioTyper DB Update-V3.3) was operated with 1 μl matrix consisting of a saturated solution of z-cyano-4-hydroxycinnamic acid in 50% acetonitrile/2.5% trifluoroacetic acid (Bruker z-cyano; Bruker Daltonics). The target slide was then loaded into the Microflex MS machine and the sample submitted to multiple laser shots.

**Measurements with the VITEK MALDI-TOF MS.** All isolates were tested in duplicate. The system was operated as described by Cherkaoui *et al.* (2010). Briefly, a portion of a colony was picked up from the anaerobic Brucella blood agar and inoculated onto a spot on the plate target slide. A ready-made matrix solution (consisting of a solution of 3.10 g z-cyano-4-hydroxycinnamic acid in 25.44 g acetonitrile, 25.57 g ethanol in 100 ml solvent) was applied to the spot on the target slide and allowed to dry. Then the slide was loaded into the VITEK MS machine. As with the Microflex MS system, the sample was submitted to multiple laser shots. The matrix absorbs the laser light and vaporizes along with the sample process of ionization. A VITEK mass spectrometer was used to generate spectra from the bacterial suspension and the Biotyper software (version 2.00) was used to analyse the results. Both systems were calibrated immediately before the analysis according to the manufacturer’s instructions.

**Quality control.** *Escherichia coli* ATCC 8739, *B. fragilis* ATCC 25285 and *C. difficile* ATCC 70057 were included as positive quality controls in each run with both systems and matrices. A negative control consisting of matrix alone with no organism was also included in each run.

**Data analysis.** The identification criteria were chosen according to the cut-offs proposed by the manufacturers. For the Bruker Microflex MS, identification interpretation was as follows: 0.00–1.699 represent no reliable identification, a score of ≥1.700 and ≤1.999 was considered as probable identification at the genus level, a score of ≥2.000–2.299 was considered as secure genus identification and probable species identification; and a score of ≥2.300–3.000 was considered as secure identification at species level. In other words, the manufacturer’s recommended score cut-offs were used to determine the genus-level (1.700–1.999) or species-level (≥2.000) identification. For the VITEK MS, the results were evaluated according to a coloured index: green for percentages ≥90%, yellow for those between 85 and 89.9%, and white for those below 85%. All of the identifications to the genus or species level fell into the green zone, with a score above 90% considered reliable. Scores between 85 and 90% were also considered as acceptable identifications. A cut-off of 90% was chosen for the VITEK MS.

**Discrepancies.** The first response to a discrepancy was to repeat the analysis using both Bruker Microflex MS and VITEK MS to eliminate the possibility of contamination. The remaining discrepancies were resolved by performing 16S rRNA gene amplification and sequencing.

**16S rRNA gene amplification, sequencing and interpretation.** DNA of the strain was isolated as described by Boom *et al.* (1990) and the 16S rRNA genes were amplified and sequenced using universal 16S rRNA-specific primers (Hiraishi, 1992). The sequences obtained were compared with sequences present in the GenBank database using BLAST software (http://www.ncbi.nlm.nih.gov).

**RESULTS**

Table 1 lists the 274 clinical isolates belonging to five genera and 14 species that were tested by both the Bruker MS and VITEK MS systems compared to API 20AN. The VITEK MS identified all isolates to the genus and species level in agreement with the API 20AN, while the Bruker MS identified 99.2% of all isolates to the genus and species level compared to API 20AN.

Both systems correctly identified all species of *Clostridium* and *Peptostreptococcus* comparable with the conventional system. VITEK MS and API 20AN were in agreement in the identification of all *Bacteroides* spp., including *B. fragilis* and *B. thetaiotaomicron* isolates.

The Bruker MS could identify 85.5% of the anaerobes to genus level, including probable genus identification (48.5%) and highly probable genus identification (40%). Log scores of eight isolates (one *B. vulgatus*, 1.68; one *Prevotella bivia*, 1.179; one *B. ovatus*, 1.154; one *C. difficile*, 1.687; one *C. sporogenes*, 1.629; one *B. thetaiotaomicron*, 1.687; one *B. fragilis*, 1.419; and one *Peptostreptococcus asaccharolyticus*, 1.461) were <1.7 in the Bruker MS, i.e. unreliable identification. However, they were correctly identified at the genus and species level according to the identification by 16S rRNA sequencing. Another 22 (8%)
isolates [three Prevotella bivia, one B. ovatus, two C. perfringens, two C. histolyticum, ten C. difficile, two B. vulgatus, two B. fragilis] gave scores between 1.7 and 1.999, meaning they could be validated only to genus level, but with sequencing, their identification was correct at the genus and species levels.

Table 2 shows the sequencing results of those isolates that gave discrepant results between the Bruker MS, VITEK MS and API 20AN. Bruker MS misidentified one B. fragilis and one B. thetaiotaomicron isolate as Malika spinosa (score 1.393) and Propionibacterium acnes (score 1.464), respectively. The two discrepant results were resolved by 16S rRNA sequencing in favour of VITEK MS and API 20AN.

Bruker identified 244 (89.1 %) and 28 (10.2 %) isolates to the species and genus level, respectively. VITEK MS identified 247 (100 %) isolates to species level. The Bruker MS misidentified only 2/274 (0.72 %) of the isolates, compared to none misidentified by the VITEK MS.

**DISCUSSION**

Conventional identification methods for anaerobes are cumbersome, time consuming and need specific anaerobic environment. MALDI-TOF MS has now been used and implemented in some laboratories for efficient, rapid and cost-effective identification of different classes of bacteria, including anaerobes. The correct identification of an organism is dependent on the presence of the reference strains in the database because the species of the reference strain will give the closest match for the identification of the tested strain. In our study, more isolates could be identified to the species level with the VITEK MS system: 100 % versus 89.1 % by the Bruker MS. This is similar to a recent report by Veloo et al. (2011b), where the corresponding numbers were 61 % with the Shimadzu/SARAMIS system (old name for VITEK MS) and 51 % with the Bruker MS system. However, this is in contrast to a recent paper by Justesen et al. (2011), where the corresponding number was 43.8–49 % for the Shimadzu/SARAMIS system and 67 % for the Bruker MS system. Although the Bruker system gave an unreliable score (<1.7) for 8/247 (2.9 %) isolates, the final identification was accurate compared to the conventional system and sequencing. Likewise, a score between 1.7 and <2.0 was given to 22/274 (8 %) isolates, i.e. the Bruker MS gave the correct identification to both genus and species level, but according to the manufacturer’s instructions, we can depend on their identification to the genus level only. This is in contrast to a recent report by Nagy et al. (2012), who could identify 218/283 (77 %) isolates to species level (with score ≥2.0) and 31/283 of isolates (10.95 %) to genus level (with score 1.7–2.0) and 34 isolates (12 %) gave no reliable identification (score <1.7). Another group (Schmitt et al., 2012) found that correct genus identification could be achieved for 57 % (120/209) of anaerobes with score >2.0, and correct species identification was achieved for 80 % (168/209) of the isolates.
The identification of anaerobes by MS offers several advantages in comparison with the conventional routine method. Shortening the time period required to identify an organism from days to a few minutes will improve the clinical outcome for the patients (Cherkaoui et al., 2010). There is a great and significant impact on time to identification of biochemically inert, fastidious and slow-growing anaerobic cocci (Tan et al., 2012). It is justified to use MALDI-TOF MS for the identification of anaerobes in our laboratory, where more than one-third (113/274; 41%) of our isolates are Bacteroides species. This is related to the fact that the MALDI-TOF MS protocol can reduce reagent use and labour costs significantly (Tan et al., 2012). The strength of our study is the implementation of MALDI-TOF MS in the routine setting with comparison of the conventional system on clinical isolates and the use of 16S rRNA sequencing for analysis of discrepancies. However, one limitation of our study is the small number of genera and species that were isolated and tested; certain species, e.g., Finegoldia magna and Parabacteroides species, were not tested because of the small number of mixed anaerobes isolated during the study period, most of which were considered as part of the mixed normal flora and were thus not identified further.

One of the drawbacks of MALDI-TOF is that it requires cultured organisms rather than direct clinical specimens. In addition, the available database of the Bruker MS needs to be optimized for routine identification of anaerobes, as some organisms could not be identified by this system (Veloo et al., 2011a, 2011c). The Bruker MS has been evaluated for identification of organisms directly from blood culture but currently does not provide data about antimicrobial susceptibility patterns.

In conclusion, MALDI-TOF is a rapid, simple, inexpensive technique, user friendly (VITEK MS more so than Bruker MS), and with a relatively small-sized machine (Bruker) that can be incorporated into the routine diagnostic laboratory and used for the identification of anaerobes. It can easily be implemented in the routine conventional laboratory.

### REFERENCES


### Table 2. API 20AN identification, VITEK MS and 16S rRNA sequencing data of isolates for which mismatched and no reliable identification was obtained by Bruker MS (i.e. score <1.7)

<table>
<thead>
<tr>
<th>Species ID and score obtained by API</th>
<th>Species ID and score obtained by Bruker</th>
<th>Species ID and score obtained by VITEK MS</th>
<th>16S rRNA sequencing result</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. fragilis (99.9)</td>
<td>Malika spinosa (1.393)</td>
<td>B. fragilis (99.9)</td>
<td>B. thetaiotaomicron</td>
</tr>
<tr>
<td>B. ovatus/thetaiotaomicron (99.9)</td>
<td>Propionibacterium acnes (1.464)</td>
<td>B. thetaiotaomicron (99.9)</td>
<td>B. fragilis</td>
</tr>
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