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# Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry for identification of *Clostridium* species isolated from Saudi Arabia

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ABSTRACT

The aim of this study was to identify different *Clostridium* spp. isolated from currency notes from the Ha'il region of Saudi Arabia in September 2014 using MALDI–TOF-MS. *Clostridium* spp. were identified by Bruker MALDI–TOF-MS and compared with VITEK 2. The confirmation of the presence of different *Clostridium* spp. was performed by determining the sequence of the 16S ribosomal RNA gene. In this study, 144 *Clostridium* spp. were isolated. Among these specimens, MALDI–TOF-MS could identify 88.8% (128/144) of the isolates to the species level and 92.3% (133/144) to the genus level, whereas, VITEK 2 identified 77.7% of the (112/144) isolates. The correct identification of the 144 isolates was performed by sequence analysis of the 500 bp 16S rRNA gene. The most common *Clostridium* spp. identified were *Clostridium perfringens* (67.36%), *Clostridium subterminale* (14.58%), *Clostridium sordellii* (9%) and *Clostridium sporogenes* (9%). The results of this study demonstrate that MALDI–TOF-MS is a rapid, accurate and user friendly technique for the identification of *Clostridium* spp. Additionally, MALDI–TOF-MS has advantages over VITEK 2 in the identification of fastidious micro-organisms, such as *Clostridium* spp. Incorporating this technique into routine microbiology would lead to more successful and rapid identification of pathogenic and difficult to identify micro-organisms.

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## Introduction

Many *Clostridium* spp. are capable of causing invasive infections in humans, some of which could be serious and life threatening, such as myonecrosis and bacteremia. The ability of *Clostridium* spp. to cause serious infections results predominantly from the production of harmful toxins.[1] Production of potent toxins by *Clostridium* spp., particularly by *C. botulinum*, *C. perfringens*, *C. tetani* and *C. difficile*, leads to severe diseases such as botulism, gas gangrene, tetanus and pseudomembranous colitis.[2–4] *Clostridium* spp. are fastidious in nature, and their isolation, culture and identification in a routine diagnostic microbiology laboratory are complicated and

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time consuming. There are several reasons that the identification of *Clostridium* spp. in a routine microbiology laboratory is difficult, including the requirement for a specific anaerobic system, such as an anaerobic jar for the culture, an extended incubation period, and an occasional loss of isolates during subculture because of oxygen sensitivity. Phenotypic and biochemical methods require time because the procedures are lengthy, and at times, they fail to distinguish between closely related spp. PCR-based molecular methods and sequencing are expensive and difficult to use for routine diagnostic procedures, and they require committed technical expertise.[5]

Recently, many technological improvements to methods for the identification of micro-organisms, such as MALDI–TOF-MS, have successfully been incorporated in microbiology laboratories globally. Compared with conventional methods, MALDI–TOF-MS is a useful, rapid, accurate and simple technique for the correct identification of micro-organisms.[6] Several studies have highlighted the advantages and performance of MALDI–TOF-MS including, rapidity, low sample volume requirements and low reagent costs compared with currently available methods. Many studies using this technology, which is predominantly used for the identification of aerobic bacteria, have led to this technology being used in many clinical laboratories worldwide. Very few studies have been conducted on the use of MALDI–TOF-MS to identify anaerobic bacteria. The aim of this study was to identify the *Clostridium* spp. obtained from currency notes in Saudi Arabia using MALDI–TOF-MS.

## Materials and methods

### *Study design and bacterial isolation*

In this study, 144 *Clostridium* spp. were isolated in sterile tubes from 320 currency notes (1-Riyal) collected separately from the Ha'il region in September 2014. The notes were collected in sterile tubes to avoid cross contamination and then transferred into new sterile tubes containing sterile brain heart infusion (BHI) broth. The tubes were vortexed for 30 s followed by incubation in a shaker incubator for 4 h at 37 °C. The tubes were vortexed again for 30 s and incubated at 37 °C overnight. The samples were sub cultured on blood agar (BA) plates containing 50 μg of metronidazole and 10 μg gentamicin discs (Oxoid, UK). The plates were incubated for 48–72 h at 37 °C using anaerobic jars (Oxoid, UK). All colonies that were susceptible to metronidazole and resistant to gentamicin were selected and sub cultured on two separate blood agar plates and incubated aerobically and anaerobically. All isolates that grew anaerobically and not aerobically were designated anaerobic bacteria and were selected for further identification.

### *Identification of bacterial isolates by MALDI–TOF-MS*

Isolates were identified by MALDI–TOF-MS (Bruker Daltonics, Bremen, Germany) using a formic acid-based direct, on-plate preparation method.[6] In this method, one microliter of 70% formic acid per well was deposited onto the MALDI–TOF MS steel anchor plate (BigAnchor 96-well plate; Bruker Daltonics). The colonies were spread into the formic acid and allowed to dry. The dried mixture was overlain with 2 μl of matrix solution (α-cyano-4-hydroxycinnamic acid (HCCA); Bruker Daltonics), dissolved in 50% acetonitrile, 47.5% water, and 2.5% trifluoroacetic acid and allowed to dry prior to analysis using a MALDI Biotyper. A MicroFlex LT mass spectrometer (Bruker Daltonics) was used for the analysis. The spectra were analyzed using Bruker Biotyper 3.0 software. The manufacturer-recommended cutoff scores were used for identification, with scores of ≥2.000 indicating identification to the species level, scores between 1.700 and 1.999 indicating identification to the genus level, and scores of <1.700 indicating no identification. The isolates producing scores of <1.700 were retested once, and the highest score was used for the final analysis.

### *Identification of bacterial isolates by VITEK 2*

Additionally, the bacterial isolates were identified using a VITEK 2 (bioMérieux, France) according to the manufacturer's guidelines for anaerobic identification.

### *Identification of bacterial isolates by the 16S rRNA gene sequence*

The identification of the isolates was performed with the 16S rRNA gene sequence. The DNA of the bacterial isolates was extracted, and amplification of the 510 bp of 16S rRNA gene was performed according to the previously described method, using the universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 519R (5′-GWATTACCG-CGGCKGCTG-3′).[7] The sequencing of the 510 bp PCR product was performed using the above forward and reverse primers on an Applied Biosystems 3500 Genetic Analyzer according to the manufacturer's instructions. The sequence results were analyzed using GenBank (http://www.ncbi.nlm.nih.gov).

## Results

The identification of different *Clostridium* spp. from our study using MALDI–TOF-MS is presented in Table 1. The results showed that of 144 *Clostridium* spp., MALDI–TOF-MS could correctly identify 88.8% (128/144) to the species level and 92.3% (133/144) to the genus level. The *Clostridium* spp. identified at the species level and genus level were as follows: (i) *C. perfringens*, 94.8% (92/97) of the isolates were identified to the species

**Table 1 – Identification of 144 *Clostridium* species using MALDI–TOF-MS at a log score ≥2.000 (species level) and ≥1.700 (genus level).**

| Species | MALDI–TOF MS at log (score) | | | |
|---|---|---|---|---|
| | ≥2.000 | ≥1.700 | Not reliable | Misidentification |
| *C. perfringens* | 92 | 95 | 2 | 0 |
| *C. subterminale* | 17 | 17 | 0 | 4 |
| *C. sordelli* | 7 | 8 | 1 | 4 |
| *C. sporogenes* | 12 | 13 | 0 | 0 |
| Total | 128 | 133 | 3 | 8 |

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