Comparative MALDI-TOF analysis of *Staphylococcus spp.* from the environment including antibiotic resistant strains

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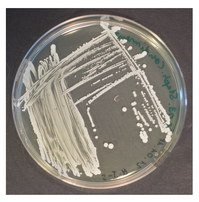
Introduction

MALDI-TOF MS is being used in clinical microbiology to rapidly identify bacteria to the species level. To achieve this requires a preassembled database of microbial mass spectral profiles[1]. Several manufactures produce MALDI-TOF MS but the limitation of assembling a comprehensive database has restricted its commercial development currently to two major companies.

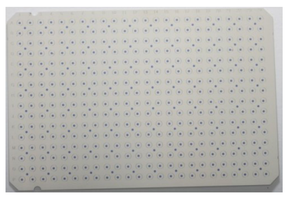
Parallel studies with clinical isolates using Bruker's Biotyper and the Shimadzu's MALDI MS using the SARAMIS system of bioMérieux has shown good concordance when reference spectra are present in the database. However, while the degree of confidence of a result largely depends on the MALDI MS and database, it is also influenced by sample preparation and even the individual worker[2]. Despite these shortcomings bacterial identifications are in good agreement suggesting that in the future it may be possible to establish an open online global database similar to those used for genome analysis. To achieve this, it may be necessary to have a universal target plate that can be used for different MALDI-MS platforms.

This study explores this principle by using a single target plate that is compatible with two instruments for parallel studies on microbial identification. We utilized the µFocus MALDI Plate 2000 µm on ASTA's new Tinkerbell LT MS and Bruker's Autoflex MS. A selection of reference bacterial isolates from the National Collection of Type Cultures (NCTC) was used to compare interspecies reproducibility while 92 environmental *Staphylococcus spp*. from 10 species, including antibiotic resistant isolates, were analysed for intraspecies compatibility.

Method



Colonies from agar plate were directly transferred to target plate



- 1 µl of 70% formic acid added to colony on target pate.
- 1 µl α-cyano-4-hydroxycinnamic acid (HCCA) used for matrix solution.

Sample analysed on µFocus MALDI Plate 2000 µm

Bruker's Autoflex

Same target plate and samples used on both MALDI-TOF MS in parallel .

ASTA'S Tinkerbell

- Nine references strains from NCTC were analysed as well as 92 environmental staphylococci isolates belonging to 10 different species.
- Environmental isolates were collected from high-frequency hand touched surfaces from general public settings and public areas within hospitals from East and West London.

Brucker Autoflex MS parameters

- Operating mode- linear positive ion.
- Ion extraction voltage- 19.5 kV.
- Laser shots – 1000 for final spectrum.
- Frequency- 200 Hz.
- Mass range –m/z 200-20, 000 Dal.

ASTA Tinkerbell LT MS parameters

- Operating mode-linear positive ion.
- Sample voltage- 18 kV.
- Laser shots 1200 for final spectrum.
- Delay time – 1100 nsec.
- Mass range- m/z 200-20,000 Dal.

Results

NCTC cultures were all identified correctly using the Bruker Autoflex with a high degree of confidence while two species were misidentified using the less comprehensive database of ASTA Tinkerbell LT (Table 1). For the environmental staphylococcal isolates the majority of species was predicted to a high confidence using the two MS platforms except for *S. aureus and S. sciuri* on ASTA and *S. cohnii* on Bruker (Table 2). Spectral data from environmental staphylococci were shown to have comparable mass ions peaks for the same samples on both instruments. Interestingly, samples that had low confidence or incorrect identification on one instrument but had high confidence or correct identification on the other mass spectrometer also followed this pattern (Figure 1).

Table 1: NCTC culture collection score

Species	Bruker score	ASTA score
Corynebacterium ulcerans	2.538	186
Escherichia coli	2.396	170
Enterococcus faecalis	2.5	188
Klebsiella pneumoniae	2.267	133
Mannheimia haemolytica	2.103	96
Morganella morganii	2.413	161
Pseudomonas aeruginosa	2.359	125
Salmonella sp	2.14	196
Staphylococcus aureus	2.14	152

Key

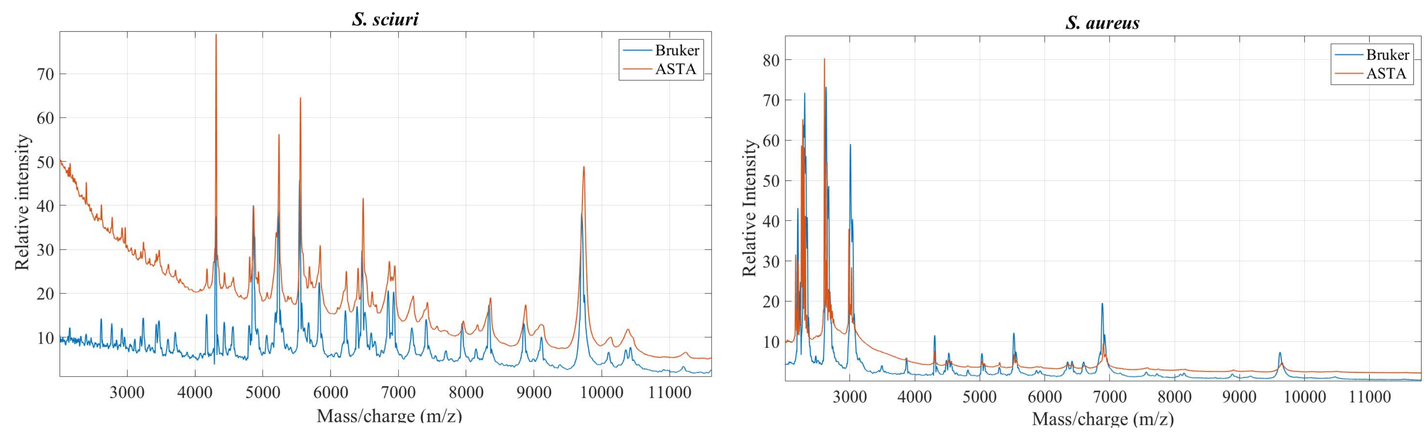
Brucker
- 2.3-3.0 = highly probable species ID
- 2.0-2.299 = secure genus ID, probable species ID
- 1.7-1.999 = probable genus ID
- 0-1.699 = not reliable ID

ASTA
- 140 ~ = fine
- 110 ~= Need to check
- 0 ~ 110 =Invalid Identification not reliable ID

Table 2: Percentage of 92 environmental staphylococci samples which was correctly identified by two MALDI-TOF instruments

Species	No isolate	Bruker		ASTA	
		% High confidence	% Correct ID	% High confidence	% Correct ID
S. aureus	8	95.84	95.84	37.5	91
S. capitis	10	96.67	100	100	100
S. cohnii	10	3.34	86.67	80	80
S. epidermidis	11	100	100	100	100
S. haemolyticus	11	90.9	100	90.9	100
S. hominis	11	100	100	96.97	100
S. pasteuri	2	83.34	83.34	66.66	100
S. sciuri	6	88.89	100	0	55.56
S. saprophyticus	12	100	100	100	100
S. warneri	11	90.91	100	93.94	96.97

Figure 1: Comparison of spectra of the species that had a low confidence or incorrect identification on one instrument but had high confidence or correct identification on the other MS platform



Discussion

- With few exceptions, there was excellent congruence between data derived using ASTA's Tinkerbell LT and Bruker's Autoflex at a species level.
- Spectra between the two instruments were shown to have key mass ions even for samples that were identified as different species or their level of confidence in identification between the two instruments were different, suggesting the absence of a reference spectrum
- The only limitation in species identification was due to differences in databases of each manufacturer.
- During the past decade the focus on assembling MS databases was for use in clinical microbiology and environmental isolates were not considered a priority. However, the boundaries between clinical and environmental sites is often blurred and so gradually more environmental isolates were incorporated in databases for taxa such as mycobacteria, staphylococci, streptococci etc
- We envisage that in the future, the need for each company to maintain and develop its own database will become too cumbersome and, out of necessity, will give way to an open online global database. This will enable analyses of isolates from varied and diverse sites around the globe whose spectra may be retained and used as reference spectra for the study of new habitats.

References

1. Veloo ACM., Jean-Pierre H, Justesen US, Morris T, Urban E, Wybo I, Shah HN, Friedrich AW, Nagy E, Kostrzewa M. (2017). A multi-center ring trial for the identification of anaerobic bacteria using MALDI-TOF MS. Anaerobe. **48**; 94-97

Keys CJ, Dare DJ, Sutton H, Wells G, Lunt M, McKenna T, McDowall M, Shah HN (2004) Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterisation of bacteria implicated in human infectious diseases. *Infection, Genetics and Evolution*: **4**, 221-242.