Introduction
Staphylococcus haemolyticus is an emerging pathogen more widely recognized for its virulence than for MRSA. It is isolated from miscellaneous soft-tissue infections, burn wounds, endocarditis, meningitis, surgical site infections, and respiratory infections. It may mistakenly be perceived and identified as low-grade MRSA infection. Analysis of these isolates is critical for monitoring and controlling the spread of infections and defining new strategies for high sample throughput and epidemiological investigation. As a result of this study, a MALDI-HDFP database was established, allowing for the rapid identification of clinical isolates of S. haemolyticus. The MALDI-HDFP technique allows high throughput and rapid identification of isolates, since the collection and analysis of datasets can be used to calibrate and optimize high sample throughput - time experiments.

Methods
Staphylococcus haemolyticus cells from a NSW hospital were isolated and cultured on Columbia Blood agar (CBA) containing 5% (v/v) horse blood, in accordance with NCTC guidelines, ‘Opening of Ampoules’. Cultures of each strain were maintained by sub-culturing on CBA and transferring to microaerobic conditions using an enriched carbon dioxide atmosphere (95.2% enriched carbon dioxide; 5.2% enriched oxygen; 95.2% enriched nitrogen). 1. Each isolate was inoculated onto two Columbia Blood agar (CBA) plates containing an enriched carbon dioxide atmosphere (95.2% enriched carbon dioxide; 5.2% enriched oxygen; 95.2% enriched nitrogen). 2. A display of the tabulated results, the test spectrum and database matches were conclusive for the 12 replicate spectra; and the similarity of the database and test spectra. Therefore filtering the top results for each strain was performed for the distinctive mass peak in the mass range 800–3,000 Da.

Preparation of bacterial samples for MALDI-TOF MS analysis

1. Using a 1 µL loop, bacteria free volumes from the plate were transferred to a MALDI-TOF MS target (Bruker Daltonics GmbH). 2. The top 8 database matches are tabulated in order of probability. 3. Samples were overlaid with 1 µL aliquot of a saturated matrix solution; 4. Test strains were cultured as for the “Peterborough Collection” initially from frozen storage and subsequently from current collection of S. haemolyticus isolates.

Analysis of clinical isolates using the Staph API system (BioMerieux, Marcy L’etoile, France) was performed in accordance with the manufacturer’s instructions. Outliers were excluded from addition to the database.

Database construction

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Database matching for distinctive mass peak was performed in the mass range 800–3,000 Da. The top results for each strain were subjected to repetitive MALDI-TOF MS analysis, increasing the reproducibility using the root mean square (RMS) value; this value is a statistical parameter used to indicate the deviation of a set of results from the mean value. Correct first match generally produced more intense spectral peaks representing the test strain. Database matches were conclusive for the 12 replicate spectra; and the similarity of the database and test spectra. Therefore filtering the top results for each strain was performed for the distinctive mass peak in the mass range 800–3,000 Da.

Identification of Clinical Isolates is possible using MALDI-TOF MS

• The database retains high and reliable spectrum identification.

MALDI-HDFP has successfully identified 97.8% of the study S. haemolyticus isolates, while MALDI-TOF MS was able to identifiy 94.4% of the study S. haemolyticus isolates.

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