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TECHNICAL NOTE

Rapid discrimination between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* by intact cell mass spectrometry

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Rapid, accurate discrimination between methicillin-sensitive Staphylococcus aureus (MSSA) and methicillin-resistant S. aureus (MRSA) strains is essential for appropriate therapeutic management and timely intervention for infection control. A rapid method involving intact cell mass spectrometry (ICMS) is presented that shows promise for identification, discrimination of MSSA from MRSA and typing. In ICMS, cells from a bacterial colony are emulsified in a chemical matrix, added to a sample slide, dried and analysed by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). This technique examines the chemistry of the intact bacterial cell surface, yielding spectra consisting of a series of peaks from 500 to 10 000, which represent the mass:charge (m:z) ratios. Each peak corresponds to a molecular fragment released from the cell surface during laser desorption. Specimens can be prepared in a few seconds from plate cultures and a spectrum can be obtained within 2 min. ICMS spectra for 20 staphylococcal isolates showed characteristic peaks, some of which were conserved at species level, some at strain level and some were characteristic of the methicillin susceptibility status of the strain. ICMS may have potential for MRSA identification and typing, and may improve infection control measures.

Introduction

Staphylococcus aureus is one of the most commonly isolated pathogens [1], accounting for c. 46% of infections in intensive care units [2] and hospital outbreaks of methicillin-resistant S. aureus (MRSA) are a worldwide problem. Rapid differentiation of MRSA from methicillin-sensitive S. aureus (MSSA) is essential for appropriate therapy and timely intervention for cross-infection control, but conventional methods are time-consuming and costly [3]. Subtyping of MRSA in putative outbreaks increases costs and adds further delay. The emergence of epidemic MRSA (EMRSA), such as types 15 and 16 in England and Wales [4], increases the requirement for fine discrimination in typing. Molecular subtyping methods, e.g., pulsed-field gel electrophoresis (PFGE), provide finer discrimination than the traditional phage-typing technique, but take several days to perform [5]. Although rapid methods to detect methicillin resistance based on the mecA PCR have been developed [6], there are problems. The mecA gene occurs in some coagulase-negative staphylococci (CNS) and in some strains of S. aureus that are phenotypically methicillin sensitive [7].

Mass spectrometry has been used widely for identification and typing of micro-organisms [8, 9]. Early work involved solvent extraction and chemical modification before analysis, but some methods use simple specimen preparation and direct analysis via appropriate ionisation methods [10]. Fast atom bombardment, electron impact and electrospray ionisation are extensively used for analysis of solvent extracts or volatile derivatives [11, 12]. Prior separation of components of mixtures by chromatographic procedures, sometimes on-line by direct coupling to the mass spectrometer, has also been employed [13]. Until recently [14], whole-cell analysis has centred on pyrolysis mass spectrometery [15]. All these approaches have drawbacks: complicated sample preparation or extraction procedures,

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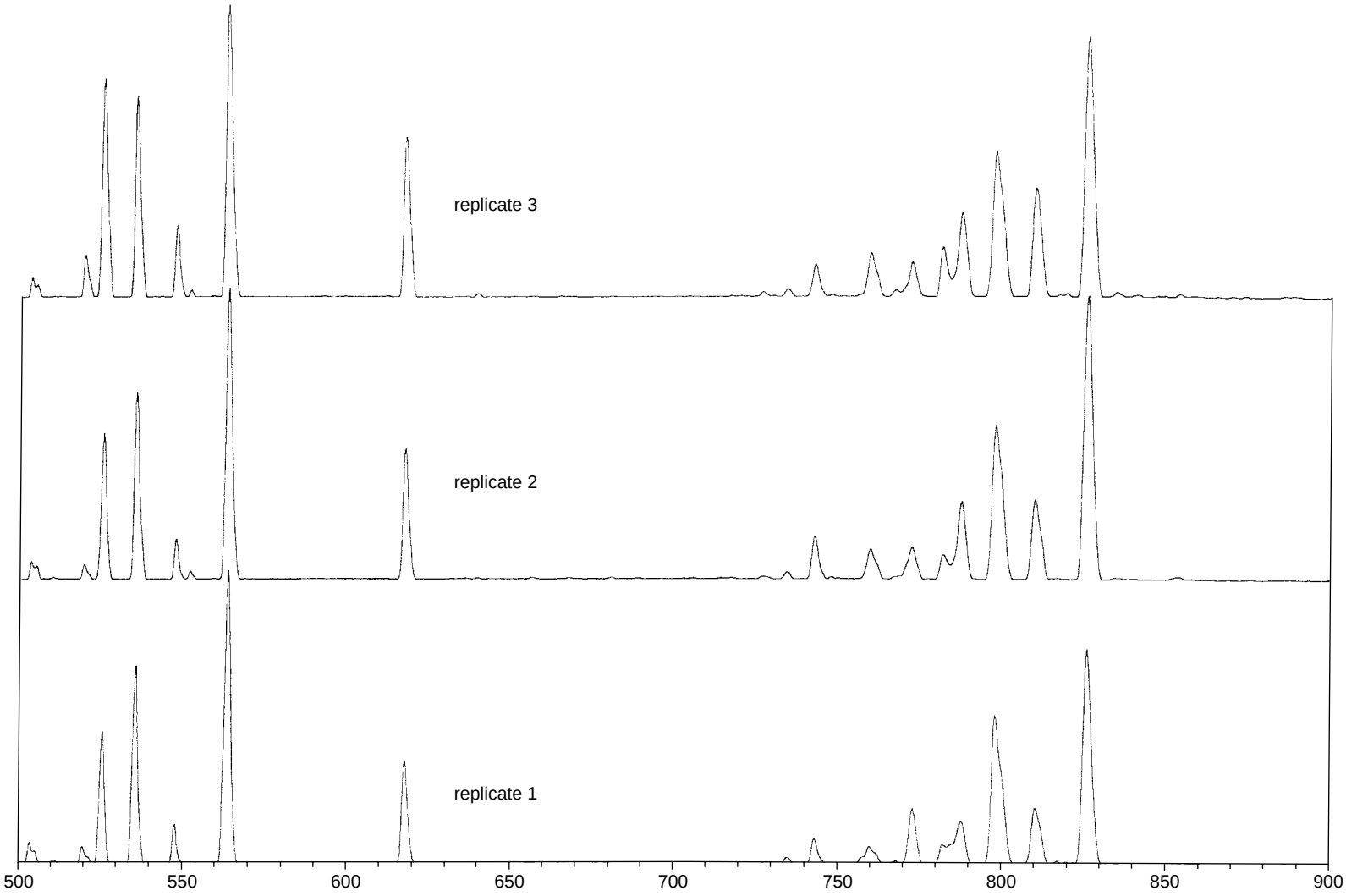

Fig. 1. The spectra obtained from three replicates of S. aureus NCIMB 8625.

complex chemical derivatisations, cumbersome data analysis or poor inter-batch reproducibility.

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) is an established technique for profiling bacterial proteins from cell extracts [16]. Recently, this technique has been applied to intact bacterial cells technique termed intact cell mass spectrometry (ICMS), providing a reproducible spectrum within minutes [14, 17]. Bacterial cells are applied to a sample slide, matrix is added and the slide is transferred to the mass spectrometer. Here, a laser beam vaporises the matrix, which carries away and ionises large mol. wt. (500–10 000) fragments from the cell surface into the vapour phase. These ions are extracted into a drift tube, where they separate and are sequentially delivered to a detector in order of their mass:charge (m:z) ratio. Negative and positive ions are produced and either can be analysed. This report summarises preliminary experiments in which ICMS was applied to discrimination between MRSA and MSSA.

Materials and methods

Strains comprised: seven strains of MSSA (ATCC 13301, ATCC 25923, NCIMB 11195, NCIMB 6571, ATCC 27659, NCIMB 8625 and NCIMB 11852); seven hospital isolates of MRSA that had been typed by PFGE and conventional phage typing (provided by Manchester Public Health Laboratory, Withington Hospital, Manchester); and six strains of CNS (S. saprophyticus NCIMB 8711, a clinical isolate of S. warnerii, S. cohnii NCTC 11041, S. haemolyticus NCTC 11042, S. epidermidis NCTC 7944 and S. epidermidis NCTC 11047). These were cultured on Columbia Blood Agar (CBA; Lab M, Wash Lane, Bury, Lancs) with horse blood (HBO 34; TCS, Botolph Claydon, Bucks) 5%, incubated overnight at 37°C.

A single colony was removed with a sterile plastic loop and smeared on to the appropriate segment of a sample slide (Kratos Analytical, Salford, Lancs), taking 10 replicates of each isolate. Matrix solution (1 μl of a saturated solution of 5-chloro-2 mercapto-benzothiazole dissolved in a water:methanol:acetonitrile 1:1:1 mix, containing formic acid 0.1% and 0.01 M of 18 Crown-6 ether) was added to each sample and allowed to dry.

Samples were analysed with a Kompact MALDI 2 linear, time-of-flight mass spectrometer (Kratos Analytical), desorbing with a 3-ns pulse of nitrogen laser light (337 nm wavelength) of intensity just above the threshold for ion production. Positive ions were extracted with an accelerating voltage of +20 kV [13]. Spectra for individual specimens were compiled, averaging results from 100 shots taken across the width of the specimen for m:z values 500–10 000. Spectra for the 10 replicates were compared to determine within-isolate peak reproducibility, then summed to minimise random effects of baseline drift and noise. Only peaks with intensities >0.4 mV after baseline subtraction were considered in the analysis. The presence and absence of peaks were considered as fingerprints for the particular isolate.

Table 1. Summary of the m:z values found in all staphylococcal isolates tested

m:z value	All staphylococci	MSSA	MRSA	Other species
511			511	511 sco, sha, ssa
525	525			
552				552 sco, sep, sha, ssa
563			563	563 sco, sep, sha, ssa
617	617			
640			640	640 sco, sep, sha, ssa
734	734			
743			743	743 sco, ssa, swa
760	760			
767			767	767 swa
773			773	773 sco, sep, ssa
787	787			
798	798			
826	826			
854			854	854 swa
891			891	
999			999	999 swa
1026			1026	1026 swa
1140			1140	
1165			1165	
1229			1229	
2127			2127	
2548		2548		
2647		2647		

Numbers in bold type denote the presence of peaks in all the staphylococcal species tested, i.e., potential genus markers. sco, S. cohnii; sep, S. epidermidis; ssa, S. saprophyticus; sha, S. haemolyticus; swa, S. warnerii.

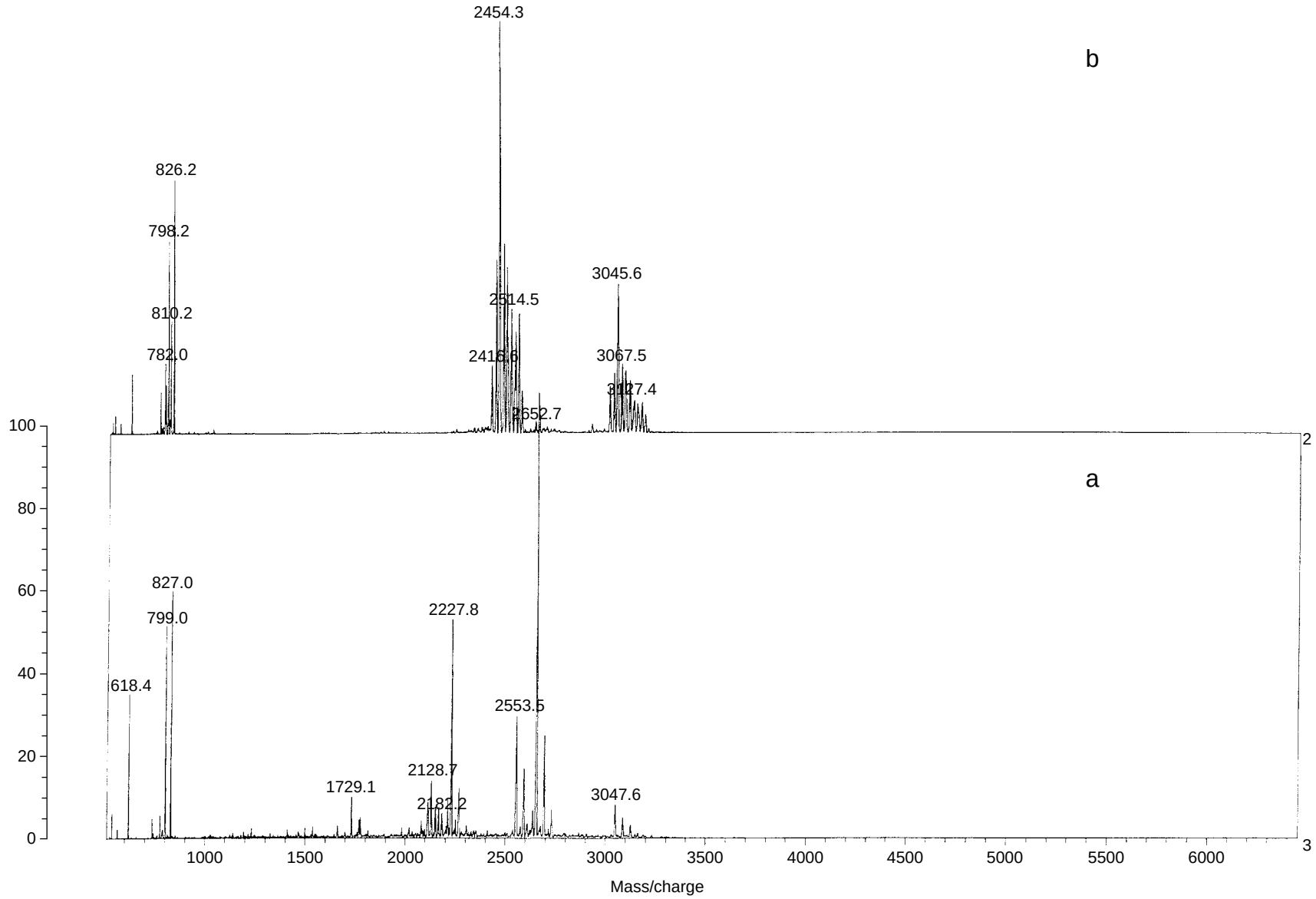

Fig. 2. Typical spectra: (a) MSSA, (b) MRSA.

Results were transferred to Excel spreadsheets and subjected to hierarchical cluster analysis by using the Dice similarity coefficient in the statistical suite SPSS for Windows.

Results and discussion

Within-strain reproducibilty was good; peaks were resolved with ±0.1% reproducibility (manufacturer's specification) on the mass:charge (m:z) scale. Over 90% of the peaks were reproduced in all replicates and relative intensities (but not absolute intensities) were closely comparable. Other studies (unpublished data) have shown little loss in reproducibility between batches analysed on different days. Most peaks were found in the m:z range 500–2000. Typical replicate spectra for MSSA (NCIMB 8625) are shown in Fig. 1.

Peak conservation is illustrated in Table 1. Some peaks (at m:z 525, 617, 734, 760, 787, 798 and 826) were present in all isolates, some were invariably present in, and unique to, MRSA (e.g., 891, 1140, 1165, 1229 and 2127) or MSSA (e.g., 2548 and 2647) and many were unique to individual strains. MSSA strains produced small numbers of peaks (37–67) and MRSA strains produced more (82–209), allowing ready visual discrimination. The spectra of the CNS showed marked inter-species differences, but could be divided into two broad groups, one with few peaks (30–48) comprising S. epidermidis and S. warneri, and the other with 92–106 peaks, comprising S. haemolyticus, S. saprophyticus and S. cohnii. Spectra were readily discriminated on visual inspection into the tight distinct MRSA and MSSA groups and a widely varied, but distinct miscellany of CNS strains.

At a finer level of discrimination, there were visually obvious differences between almost all the MRSA isolates, particularly in the peak patterns within m:z ranges 1100–1250, 1290–2040 and 3060–3220. However, two pairs of MRSA isolates were not readily distinguished in ICMS either visually or in mathematical analysis (Dice coefficient >70% similarity). These pairs were also indistinguishable by phage and PFGE typing: one pair were both EMRSA 15 PFGE type 5 and the other were both EMRSA 16 PFGE type 1. These were the only pairs among the MRSA isolates that were indistinguishable by conventional typing.

The clarity of discrimination between MSSA and MRSA strain spectra (Fig. 2) and the speed of the method suggest a potential role for ICMS in the diagnostic laboratory. Up to 10 isolates, with 10 replicates of each, could be analysed and categorised per hour. Conventional approaches that distinguish between MRSA and MSSA are slow. This introduces delays in guided treatment decisions and leaves little opportunity for the early intervention that is essential to limit the spread of MRSA. ICMS offers an opportunity to minimise the delay, and so reduce the financial and human costs of MRSA nosocomial infections. There is the additional benefit that ICMS has potential as a finely discriminatory typing tool, which is clearly pertinent to this application. From these preliminary results, ICMS spectra appear to contain data that are relevant at three levels: species identification, typing at a gross level into MRSA or MSSA, and typing at the fine level attained in PFGE. If this is so, then there are many further applications for ICMS in medical microbiology. However, there is a clear need for larger scale trials to be undertaken before these claims can be confirmed.

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