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Development of a novel matrix-assisted laser desorption/ionization time-of-flight mass spectrum (MALDI-TOF-MS)-based typing method to identify meticillin-resistant *Staphylococcus aureus* clones

O. Ueda [a,e], S. Tanaka [b], Z. Nagasawa [c], H. Hanaki [d], T. Shobuike [e], H. Miyamoto [e,*]

[a] *Microbiology Group, MM Business Unit, Siemens Healthcare Diagnostics KK, Tokyo, Japan*
[b] *Department of Clinical Laboratory, Saga University Hospital, Saga, Japan*
[c] *Department of Medical Technology and Sciences, School of Health Sciences at Fukuoka, International University of Health and Welfare, Fukuoka, Japan*
[d] *Kitasato University Research Centre for Anti-infectious Drugs, Tokyo, Japan*
[e] *Department of Pathology and Microbiology, Faculty of Medicine, Saga University, Saga, Japan*

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SUMMARY

Background: Mass spectrum analysis enables species- and subspecies-level identification, and can be used as an epidemiological tool in outbreak management. However, its reliability at clonal level has yet to be established.

Aim: To establish a matrix-assisted laser desorption/ionization time-of-flight mass-spectrum-based method that enables bacterial clone identification with accuracy equivalent to pulsed-field gel electrophoresis/phage open-reading frame typing (PFGE/POT).

Methods: Meticillin-resistant *Staphylococcus aureus* (MRSA) was used in this study. Mass spectra were obtained from a standard strain of *S. aureus* (ATCC29213) and 57 clinically isolated strains, categorized according to POT. Peaks associated with MRSA clone identification ($N = 67$) were extracted. Based on this peak information, the feasibility of MRSA clone identification was examined by cluster analysis.

Findings: In addition to the 58 strains used for peak extraction, mass spectrum analysis of 24 clinically isolated outbreak strains revealed that peak data could be used for successful identification of clones. These typing results were fully consistent with the PFGE and POT results.

Conclusion: This novel method enables simple and rapid typing with accuracy equivalent to PFGE/POT. This method would be suited to rapid outbreak analysis, offering accurate information to combat infectious diseases.

* Corresponding author. Address: Division of Microbiology, Department of Pathology and Microbiology, Faculty of Medicine, Saga University, 5-1-1 Nabeshima, Saga 849-8501, Japan. Tel.: +81 952 34 2245; fax: +81 952 34 2252.
E-mail address: miyamoth@cc.saga-u.ac.jp (H. Miyamoto).

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Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been attracting attention as a tool for microbial identification. This technique is used to identify bacterial species according to the mass spectrum patterns obtained from whole cells. This is an innovative technique that enables rapid (within approximately 30 min of bacterial culture) and accurate identification of bacterial species with simple procedures.[1–3] Peaks originating from ribosome-associated proteins that are present in large quantities in bacterial cells are mainly used for analysis. It has been reported that mass-spectrum-based analysis also enables subspecies-level identification, which is difficult using other techniques such as 16S rRNA sequencing.[4] This indicates that mass spectrum analysis could be a useful technique enabling categorization of bacterial species in further detail. In fact, the MALDI Biotyper system (Bruker Daltonik GmbH, Leipzig, Germany) has the capacity to identify various bacteria at strain or subspecies level, and its use as a device for bacterial strain identification has been established.[5–15]

Genetic-analysis-based methods have typically been used for clone-level bacterial identification, such as pulsed-field gel electrophoresis (PFGE), phage open-reading frame typing (POT), multi-locus sequence typing, *spa* typing and SCC*mec* typing. These methods have superior identification accuracy; however, their routine use is impractical because of their complicated procedures and high cost. A typing method based on patterns from drug sensitivity tests is simple; however, its accuracy is limited and it has low correlation with genetic-analysis-based typing methods.[16–19]

The identification of bacterial clones using MALDI-TOF-MS has been reported for various bacterial strains. Valerie *et al*. indicated the possibility of clone identification and meticillin-resistant *Staphylococcus aureus* (MRSA) differentiation by using several peaks located between 500 and 10,000 m/z in the mass spectra of *S. aureus*.[20] Bernardo *et al*. referred to the spectrum patterns applicable for the identification of *S. aureus* clones.[21] Furthermore, Wolters *et al*. analysed the mass spectra of five MRSA clones, selected 13 peaks for cluster analysis and identified the five clones successfully.[22]

These reports suggest the possibility of using MALDI-TOF-MS not only for species- or subspecies-level identification, but also for clone-level identification. However, previous studies have only demonstrated the validity of mass spectra for identified clones, and the applicability of the reported mass spectra for all unknown clones appears to be small. This is because a specific mass spectrum varies according to the environmental or measurement conditions, and the availability of this information specific to each clone is limited.

This study examined the mass spectra of a standard *S. aureus* strain and 57 clinically isolated MRSA strains, and extracted the peaks associated with MRSA clone identification. Cluster analysis of the extracted peak information enabled successful identification of the MRSA clones with accuracy equivalent to PFGE/POT.

Methods

Bacterial strains and media

This study included the standard *S. aureus* strain (ATCC29213) and 57 clinical isolates of MRSA. MRSA were isolated from clinical materials at Saga University Hospital in 2013. The 57 isolates were selected at random from 231 MRSA isolates stored in the hospital laboratory. Along with the standard strain, these isolates were subjected to mass spectrum analysis. In addition, as a validation test, 24 strains of MRSA (K1～K24) that were confirmed by PFGE analysis were also used. These strains were collected from outbreaks of nosocomial infections; they were isolated from five clinical institutions and maintained at Kitasato University. The strains were cultured on pourmedia sheep blood agar M70 (Eiken Chemical Co., Ltd., Tokyo, Japan) for 18 h at 35 °C. Drug sensitivity was measured using MicroScan (Pos Combo 3.1J Panel) (Siemens Healthcare Diagnostics Inc., Tokyo, Japan), and WalkAway 96 *plus* (Siemens Healthcare Diagnostics Inc.) was used in accordance with the manufacturer's instructions.

POT

Cica Geneous DNA extraction reagent (Kanto Chemical Co., Ltd, Tokyo, Japan) was used to extract DNA from the colonies. Cica Geneus Staph POT kit: *S. aureus* (Kanto Chemical Co., Inc.) was used for POT, and measurements were performed in accordance with the manufacturer's instructions. The colonies were considered to be identical when the POT coefficients for POT1, POT2 and POT3 were consistent. POT types are shown in upper-case letters.

MALDI-TOF-MS

The Microflex LT instrument (Bruker Daltonik GmbH) was used for mass spectrum analysis. Samples were pretreated with ethanol–formic acid extraction,[23] and measured using MALDI Biotyper Version 3.0 (Bruker Daltonik GmbH). Ultrapure water (300 μL) was dispensed into 1.5-mL sample tubes, and 5–10 mg of bacteria from fresh single colonies was inoculated and suspended. After mixing the cell suspension with 900 μL of ethanol (Kanto Chemical Co., Inc.), the mixture was centrifuged for 2 min at 13,000 rpm and the supernatant was removed. After resuspending the precipitate with 30 μL of 70% formic acid (Kanto Chemical Co., Inc.), 30 μL of acetonitrile (Kanto Chemical Co., Inc.) was added and mixed; thereafter, the mixture was centrifuged for 2 min at 13,000 revolutions/min (rpm) and 1 μL of the sample supernatant was used for measurement. Samples were measured in duplicate in the range of 2000–20,000 m/z with a laser frequency of 60 Hz, and integrated peaks from 240 shots were recorded (6×40 laser shots at different locations).

Analysis of mass spectrum data

FlexAnalysis software and MALDI Biotyper Version 3.02 (Bruker Daltonik GmbH) were used for comparative analysis and re-analysis of the mass spectra, respectively. ClinPro Tools Version 2.2 (Bruker Daltonik GmbH) was used for more detailed