

# Rapid Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) by the Vitek MS Saramis system

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Received: 9 June 2015 / Accepted: 5 August 2015 / Published online: 15 September 2015  
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**Abstract** A rapid, sensitive, and accurate Vitek MS assay was developed to distinguish clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical isolates of methicillin-sensitive *Staphylococcus aureus* (MSSA) by developing an in-house knowledgebase of SuperSpectra. Three unique peaks, including peaks at 2305.6 and 3007.3 Da specific to MRSA, and 6816.7 Da specific to MSSA, were selected for differentiating MRSA and MSSA. This assay accurately identified 84 and 91 % of clinical MRSA and MSSA strains out of the total 142 clinically acquired *S. aureus* strains that were tested. This method will greatly improve the efficiency of single clinical sample identification of MRSA, thereby facilitating a reduction in the transmission of MRSA in clinical settings.

## Introduction

Methicillin-resistant *Staphylococcus aureus* is among the top multidrug resistant bacterial pathogens causing nosocomial and community infections [1]. In recent years, MRSA infections have rapidly increased, and has become of serious worldwide concern [2]. After initial identification of *S. aureus*, the current conventional methods for detecting MRSA include screening techniques on solid culture medium containing oxacillin or cefoxitin, and detecting the *mecA* gene by PCR and PBP<sub>2</sub> agglutination [11]. However, these

approaches are often time-consuming, and require rather significant technical expertise, and involve rather costly instrumentation. A more rapid, accurate, and less demanding method for identifying MRSA is desperately needed.

Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) is a powerful technique for identifying microbial pathogens in the clinical laboratory [10]. The method has previously been developed to detect vancomycin-resistant *Enterococcus* (VRE) [3], rifampicin- or isoniazid-resistant *Mycobacterium tuberculosis* [5], and carbapenem-resistant *Enterobacteriaceae* [8]. Several previous studies have used the MicroFlex system (Bruker Daltonics, Germany) and Biotyper database (Bruker Daltonics, Germany) for quick identification of MRSA [7, 12]. However, as to date no studies exist in the literature reporting a valid assay to detect MRSA by using the Vitek MS system (bioMérieux, France) and the Saramis database (V.4.12, bioMérieux) in a clinical setting.

The Vitek MS system is increasingly being adopted in hospitals in China and other regions of the world for use in clinical routine as well as research practice. In this study, we aimed to establish a rapid and accurate MRSA detection method using the Vitek MS system and Saramis database.

## Materials and Methods

### Microbial Strains

Sixty-seven MRSA and sixty-six MSSA strains cultured from outpatients and inpatients in 2013 were included in this study. The strains enrolled for this study were sourced from 11 hospitals from around China, including the Second Affiliated Hospital of Zhejiang University (Hangzhou, China), the Third People's Hospital of Hangzhou City (Hangzhou, China),

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Ningbo First Hospital (Ningbo, China), The Central Hospital of Lishui City (Lishui, China), the Second Hospital of Jiaying (Jiaying, China), the First Affiliated Hospital of Wenzhou Medical University (Wenzhou, China), Zhejiang Province People's Hospital (Hangzhou, China), Sichuan Provincial People's Hospital (Chengdu, China), Henan Province People's Hospital (Zhengzhou, China), the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China), and Beijing Hospital (Beijing, China). An additional 142 *S. aureus* clinical strains collected from these hospitals in 2014 were used as validation strains. These strains were isolated from different specimens and from various hospitals widely distributed across China.

### Identification and Susceptibility Testing

Species identification of isolates was performed using the Vitek 2 automated identification system (bioMérieux). All *S. aureus* isolates were subjected to ceftiofur screening following the guidelines of the Clinical and Laboratory Standards Institute [9]. The isolates with a zone diameter of <19 mm were scored as methicillin resistant. PCR, often characterized as the 'gold standard' for MRSA detection [11] (the oligonucleotides: *mecA1*, 5'-ATGAAAAAGATAAAAATTGTTTC-3'; and *mecA2*, 5'-CTCATATGTTCTGTATT-3') was used to confirm MRSA strains.

### Vitek MS Analysis

In order to mimic routine conditions, bacteria were cultured onto Columbia blood agar (bioMérieux, France) and incubated at 35 °C overnight. Several fresh uniform colonies were resuspended into 300 µl of distilled water, and 900 µl ethanol was then added (Sigma, USA). The extraction tube was centrifuged for 2 min at 12,000×g, the supernatant then discarded, and the bacterial pellet was resuspended in 50 µl of 70 % formic acid (Sigma, USA) and 50 µl acetonitrile (Sigma, USA), and centrifuged aged for 2 min at 12,000×g. One microliter of the supernatant was placed onto a Vitek MS target plate, then air dried and overlaid with 1 µl of ready-to-use α-cyano-4-hydroxycinnamic acid (CHCA) matrix solution (bioMérieux, France) for Vitek MS analysis. A strain of ATCC8739 *Escherichia coli* was used as control in calibration of the instrument. All isolates were confirmed to be *S. aureus* by comparing to the original, unmodified Saramis database (confidence value exceeding 80 % are considered significant).

### Construction and Validation of SuperSpectra

To construct the specific SuperSpectra for MRSA, spectra of all MRSA strains were acquired and transferred to a new folder. All spectra were compared to the original Saramis

database and kept at a maximum of 40 characteristic peaks according to the number of mass peaks matched. When at least 80 % of MRSA isolates contained these peaks, also MSSA SuperSpectra. The detailed procedure was conducted according to the VITEK® MS Plus User Guide. After constructing the SuperSpectra for MRSA and MSSA, the spectra were activated within the system for identification of subsequent isolates for validation. This MRSA/MSSA detection assay we developed via SuperSpectra construction was validated using 142 *S. aureus* clinical strains collected in 2014 from different specimens from the various hospitals previously mentioned. After preparing each strain using the protein extraction method described above, all 142 strains were analyzed by Vitek MS, and then subjected to the SuperSpectra analysis of the newly constructed Saramis database.

## Results

### Identification and Susceptibility Testing

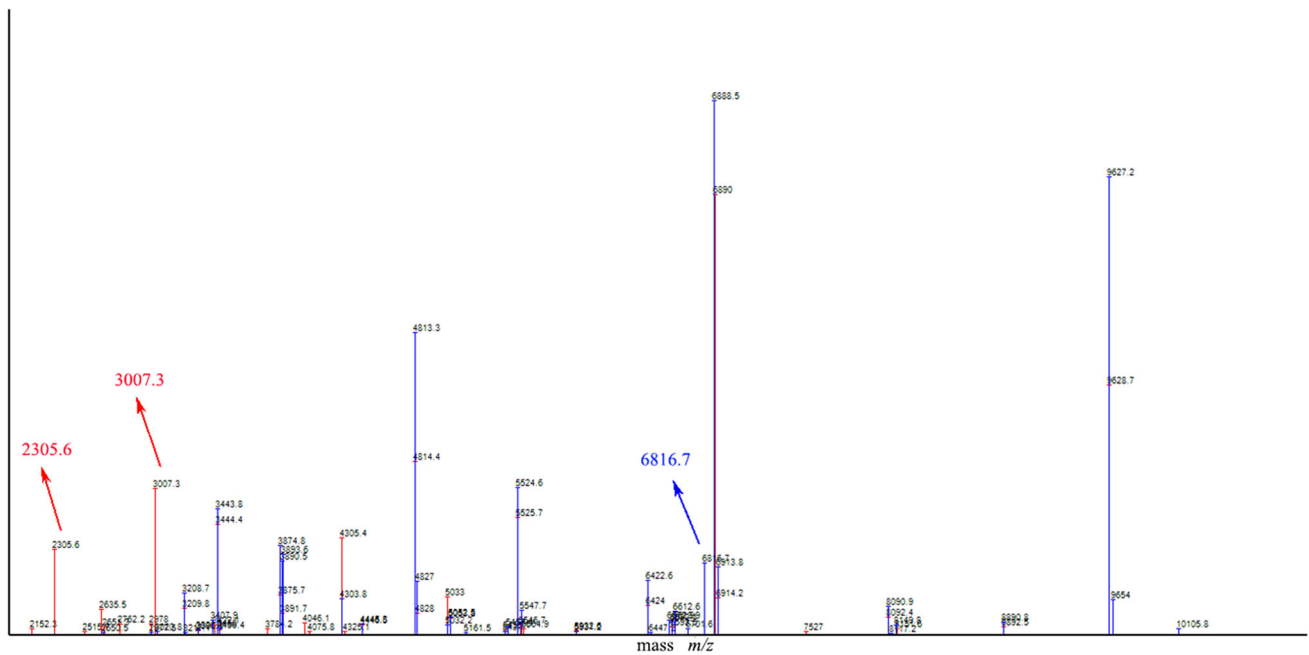
All 275 *S. aureus* isolates were collected from various specimen types from the 11 hospitals around China and confirmed by the Vitek 2 automated identification system. All 275 *S. aureus* isolates were discovered resistant to ceftiofur and positive for the *mecA* gene confirmed by PCR.

### Characteristic Peaks

There were 12 different main peaks among the SuperSpectra for MRSA and MSSA, three of which presented relatively high signal intensity (rel. intensity >5.0). Two unique peaks with a mass of 2305.6 and 3007.3 Da were detected only in the MRSA group, whereas one unique peak with a mass of 6816.7 Da was detected only in MSSA group (Fig. 1). These three specific unique peaks for MRSA and MSSA were selected in the analysis for differentiating MRSA and MSSA.

### Validation Results

Identification results by Vitek MS and conventional methods were compared to examine the accuracy and reliability of this SuperSpectra method for distinguishing MRSA and MSSA. A total of 142 clinical *S. aureus* strains (76 MRSA and 66 MSSA) confirmed by conventional. Among the 76 MRSA strains, 64 (84 %) were correctly identified as MRSA by the SuperSpectra Vitek MS assay, 10 (13 %) were mistakenly identified as MSSA, and 2 (3 %) were reported as *S. aureus* only (Table 1). Among the 66 MSSA strains, 61 (91 %) were correctly identified as MSSA, whereas 5 (7 %) were mistakenly identified as MRSA and 1 (1 %) was detected as *S. aureus* only (Table 1).



**Fig. 1** SuperSpectra of MRSA and MSSA. The 2305.6 and 3007.3 Da peaks (red) were typical spectra of MRSA, while the 6816.7 Da peak (blue) was specific spectrum of MSSA (Color figure online)

**Table 1** Results of validation of Vitek MS in identification of MRSA and MSSA in 142 *S. aureus* strains

Cultural confirmation	No. of strains	Identification by Vitek MS	Confidence values (%)	Percentage (%)
MRSA	2	<i>S. aureus</i> only	90.0–99.9	2.6
	10	MSSA(misidentification)	99.9	13.2
	1	MRSA	80.0–90.0	1.4
	7	MRSA	90.0–99.9	9.3
	56	MRSA	99.9	73.7
Subtotal	76	MRSA accuracy rate		84.2
MSSA	1	<i>S. aureus</i> only	80.0–90.0	1.5
	5	MRSA(misidentification)	99.9	7.6
	2	MSSA	80.0–90.0	3.0
	3	MSSA	90.0–99.9	4.6
	55	MSSA	99.9	83.3
Subtotal	66	MSSA accuracy rate		90.9
Total	142	Total accuracy rate		87.3

### Discussion

The mass peak at 3007.3 Da present in MRSA isolates was consistent with the expression of PSM-mec as previously reported [6], while the other two peaks were novel peaks which have never been previously identified. J.E. Goldstein et al. [4] reported that culture conditions and sample preparation prior to spectral acquisition would affect the spectrum quality of *S. aureus* spectra for MALDI-TOF MS identification. Therefore, in this study, the conditions including the extraction method and experimental environment were strictly controlled. Our results demonstrate

that the three peaks at 2305.6, 3007.3, and 6816.7 Da were the most relevant peaks as they were differentially expressed in MRSA and MSSA.

Wang. et al. [12] previously had used the Bruker Biotyper system for identification of MRSA and MSSA. According to their report, the accuracy was 65.31 and 74.29 % for MRSA and MSSA, which is much lower than the Vitek MS assay presented in this study. In addition, a larger sample size for dendrogram analysis was also needed analysis performed on the Bruker system. Therefore, this method may be not suitable for single clinical sample analysis. Taken together, the assay developed in this study

not only showed great improvement on the accuracy of MALDI-TOF MS identifying MRSA, but also this assay can also be directly used for analysis of single clinical sample, offering the advantage of simplified operation and visual result interpretation by comparing targeted mass spectra to the newly constructed SuperSpectra Saramis database.

Susceptibility testing results for all 275 *S. aureus* isolates were consistent with cefoxitin screening and *mecA* gene PCR. MALDI-TOF MS offers several advantages for detecting MRSA over cefoxitin screening and PCR: results are produced quickly at a low cost, and sample workflow and data interpretation are simplified. One limitation of the present study should be noted that 10 (13 %) MRSA strains were mistakenly identified as MSSA. However, Vitek MS used as a rapid method for predicting MRSA could be a critical tool even if the accuracy rate is not perfectly at 100 %. Out of the 142 validation strains, 3 could not be classified as either MRSA or MSSA by our SuperSpectra method. Among them, two exhibited typical intensity peaks at 2305.6 and 3007.3 Da, indicating that they should be MRSA, and these two were further confirmed as the MRSA phenotype; Another one strain was found to display spectra typical of the MSSA SuperSpectra, and was also found consistent to the classical methods of MSSA detection. It should be noted that the three strains mentioned above exhibited distinct peaks in their mass spectra which were not covered within the MRSA and MSSA SuperSpectra. Thus, further investigation will be focused on expanding the database and improving the SuperSpectra for better application within the clinical laboratory.

In conclusion, a rapid, sensitive and accurate Vitek MS assay has been developed for the identification of clinical MRSA by rigorously constructing SuperSpectra for both MRSA and MSSA. This assay will greatly facilitate rapid clinical identification of MRSA, and will reduce the transmission of MRSA in our clinical setting.

**Acknowledgments** The authors thank Shi Chen, Yibo Shi, Guoxiong Li, Xiaoyan Wu, Sufei Yu, Huoyang Lv, Kang Liao, Yi Li, Hua Yu, Qing Wu, and Yunjian Hu for providing bacteria isolates. This work was supported by Science and Technology Department of Zhejiang Province (Grant No. 2014C33191).

#### Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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