The Presence of a Single MALDI-TOF Mass Spectral Peak Predicts Methicillin Resistance in Staphylococci

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ABSTRACT

The class A *mec* gene complex also carries the *psm-mec* gene. The *psm-mec* gene product can be detected as a peak near 2415 *m/z* using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). The correlation of a 2415 *m/z* peak with methicillin resistance (*mecA* carriage) in consecutive staphylococcal blood culture isolates was evaluated. A 2415 ± 2.00 *m/z* peak was observed in 37% (51/137) of methicillin-resistant *Staphylococcus aureus*, in none (0/146) of the methicillin-susceptible *S. aureus*, in 6% (10/180) of methicillin-resistant *S. epidermidis*, and in 2% (1/63) of methicillin-susceptible *S. epidermidis* isolates. Although the sensitivity of the 2415 *m/z* peak for *mecA* carriage in *S. aureus* and *S. epidermidis* was low (37% and 6%, respectively), the specificity was high (≥98%). This peak can be identified during routine MALDI-TOF MS clinical testing, and the analysis adds no reagent cost and requires minimal time expenditure.

Keywords

MRSA, MALDI-TOF, mecA, strain typing, PFGE, antimicrobial resistance

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1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* (MRSE) isolates are commonly encountered in clinical microbiology. In addition to causing increased morbidity and mortality, MRSA has been associated with higher healthcare costs than its methicillin susceptible counterpart (Tansarli, Karageorgopoulos, Kapaskelis, & Falagas, 2013). Identifying MRSA from clinical samples more quickly and inexpensively would benefit both the laboratory and healthcare providers. The recent availability of MALDI-TOF MS has helped laboratories identify organisms more rapidly and at a lower cost (Patel, 2015). MALDI-TOF MS is routinely used only for taxonomic identification of cultured isolates, but the mass spectra contain rich data that can be used to evaluate more than simply the organism's species-level identity. For example, MALDI-TOF MS has been used to infer strain lineage, the production of virulence factors, and the presence of antimicrobial resistance (Josten, et al., 2014; Josten, et al., 2013; Ostergaard, Hansen, & Moller, 2015; Wolters, et al., 2011; Youn, et al., 2016).

Each mass spectrum is comprised of dozens of mass-charge (*m/z*) peaks. Some peaks are more discriminatory than other peaks when attempting to characterize staphylococcal isolates (Wolters, et al., 2011). Each peak correlates with a biological molecule, and one such molecule is the phenol soluble modulin, PSM-mec (Chatterjee, et al., 2011). The PSM-mec MALDI-TOF MS peak first appeared in the peer-reviewed literature in 2002, although it was not recognized as PSM-mec at the time (Du, Yang, Guo, Song, & Wang, 2002). PSM-mec was later isolated from methicillin resistant staphylococci, and its doubly-charged (1208 *m/z*) and triply-charged (806 *m/z*) ions were identified using high performance liquid chromatography followed by mass

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spectrometry (Kaito, et al., 2011; Queck, et al., 2009). Recently, PSM-mec identification in *S. aureus* using MALDI-TOF MS was thoroughly investigated (Josten, et al., 2014).

The PSM-mec peptide is coded for by the *psm-mec* gene and is present in the class A *mec* gene complex, which is carried by staphylococcal cassette chromosome *mec* (SCC*mec*) types II, III, and VIII (Chatterjee, et al., 2011); which may represent up to half of MRSA infections at some institutions (David, et al., 2014). PSM-mec does not cause methicillin resistance, and its biological is still being studied. The peptide is thought to attenuate staphylococcal virulence, and its expression can vary due to genetic variation of regulatory elements (Kaito, et al., 2013; Otto, 2014a, 2014b).

We evaluated the prevalence, specificity, and possible clinical utility of using the 2415 *m/z* peak to predict *mecA* carriage in consecutive clinical isolates *S. aureus* and *S. epidermidis*. Although multiple molecular assays for the rapid detection of *mecA* are available and often performed on blood cultures, this testing is not commonly performed for staphylococcal isolates from other specimen sources, and using MALDI-TOF MS could be a rapid and inexpensive method of identifying *mecA* carriage in some staphylococci. Rapid detection of *mecA* can decrease the time to appropriate medical management and supports antibiotic stewardship efforts. This study demonstrates that rapid detection of methicillin resistance is possible in a subset of clinically isolated staphylococci by simply evaluating data that are already produced during routine MALDI-TOF MS testing.

2. Materials and methods

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This study was performed retrospectively using data that had been generated during routine laboratory testing. The study was performed in accordance with ethical standards approved by the Cleveland Clinic Institutional Review Board (IRB #15-1641). No external funding supported this study.

2.1 Facility

The Cleveland Clinic microbiology laboratory is accredited by the College of American Pathologists, and it acts as the clinical microbiology laboratory for the health system's inpatient and outpatient services. It also serves as a reference laboratory for outside clients. At the time of this study, the laboratory was performing blood cultures for as many as seven acute care hospitals in the Cleveland Clinic system in Northeast Ohio. Over 90,000 blood cultures were performed in the laboratory in 2015.

2.2 Routine laboratory methods.

Blood cultures were collected as paired sets of an aerobic and an anaerobic blood culture bottle with approximately 10 mL of blood in each bottle. These were incubated for up to 5 days using the BacT/ALERT (bioMerieux; Durham, NC) system. Positive blood cultures containing Gram positive cocci by direct Gram stain were analyzed using the Verigene Gram-Positive Blood Culture (Verigene) Test (Nanosphere; Northbrook, IL), a microarray that includes *S. aureus*, *S. epidermidis*, and *mecA* targets (Wojewoda, et al., 2013); which served as the reference standard. The Verigene assay was chosen as the reference standard because it is the assay used in routine testing, produces objective results based on the isolate's genotype, is an FDA-cleared

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assay, and correlates well with phenotypic susceptibility testing (Wojewoda, et al., 2013). Positive blood culture broths were also subcultured to agar media, and after overnight incubation the organism was directly transferred to a target plate and identification was confirmed using a Vitek MS (bioMerieux; Durham, NC) MALDI-TOF MS system in accordance with the manufacturer's *in vitro* diagnostics (IVD) methodology. Two different Vitek MS instruments are routinely used in the laboratory. Fine tuning is performed on the Vitek MS instruments every two weeks to ensure their reliability.

2.3 Clinical isolates

We sought to obtain at least 200 isolates of each *S. aureus* and *S. epidermidis* for analysis. The study set was comprised of all *S. epidermidis* blood culture isolates with Verigene results obtained August through October 2015 and all *S. aureus* blood culture isolates with Verigene results from March through November 2015. Cultures of *S. aureus* or *S. epidermidis* in which *mecA* was detected by Verigene were considered MRSA or MRSE, respectively. Demographics, clinical characteristics, severity of disease, and diagnosis were not considered or analyzed. Polymicrobial blood cultures were excluded from this study. Only a single isolate from each subject was included in the study.

2.4 2415 m/z MALDI-TOF MS peak analysis using Vitek MS.

Mass spectra collected during routine clinical identification testing (described above) were manually examined retrospectively for a 2415 *m/z* peak by using the Vitek MS Acquisition Station software version 1.4.2b (bioMerieux; Durham, NC). The interpreters of the mass spectra

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were not blinded to the Verigene results, but the spectra were interpreted objectively in the manner described hereafter. The mass spectra were viewed by zooming into a window spanning 500 *m/z* (approximately 2200-2700 *m/z*). Prominent peaks in this window are automatically labeled by the software without user intervention. The criteria used to identify prominent peaks are incorporated into the Acquisition Station software, and these peaks are automatically labeled by the software. Only these labeled peaks were considered in the analyses. In the initial analyses, spectra containing a peak labeled as 2415 ± 4.00 *m/z* were considered positive for a 2415 *m/z* peak, which is the effective detection window described by Josten *et al* (Josten, et al., 2014). All other spectra were considered negative for the 2415 *m/z* peak. In *post hoc* analysis, spectra containing a peak labeled as 2415 ± 2.00 *m/z* were considered positive for a 2415 *m/z* peak, and all other spectra were considered negative.

2.5 USA strain characterization

A set of 12 well characterized *S. aureus* strains (USA100-USA1200 strains) were tested for the presence of the 2415 *m/z* peak using both the Vitek MS (methods described above) and the Bruker MicroFlex (Bruker Daltonics, Billerica, MA). Colony growth from agar medium was directly transferred to a target plate. Mass spectra were generated using the Bruker Biotyper system after calibration with Bacterial Test Standard (BTS) (Bruker Daltonics, Billerica, MA). After generating the mass spectra, flexAnalysis software version 3.4 (Bruker Daltonics, Billerica, MA) was used to perform smoothing and baseline subtraction on the spectra, and flexAnalysis was used to subjectively evaluate the spectra for the presence or absence of the 2415 *m/z* peak. Clinical isolates were not tested using the Bruker system.

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139 *2.6 Statistical analysis*

140 The MedCalc online statistical software (Version 16.2.1) "diagnostic test evaluation
141 calculator" was used to calculate sensitivity, specificity, positive and negative predictive values,
142 and exact Clopper-Pearson confidence intervals ("Diagnostic test evaluation calculator," 2016).

143

144 **3. Results**

145 This study examined 283 consecutive *S. aureus* and 243 consecutive *S. epidermidis* blood
146 culture isolates from unique subjects. Verigene identified 56% (137/283) of the *S. aureus* isolates
147 as MRSA and 74% (180/243) of the *S. epidermidis* isolates as MRSE. The 2415 ± 4.00 *m/z* peak
148 (Figure 1) was identified in 39% of the MRSA isolates (Table 1) and 6% of the MRSE isolates
149 (Table 2). In both *S. aureus* and *S. epidermidis*, the specificity of the presence of a 2415 ± 4.00
150 *m/z* peak for the detection of the *mecA* gene was 97%.

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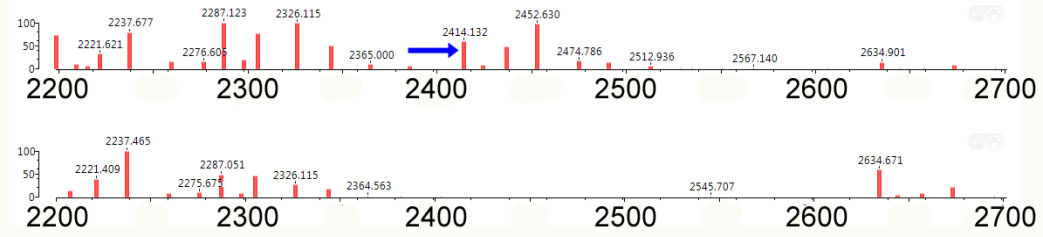

Figure 1

The Vitek MS Acquisition Station software depicts MALDI-TOF mass spectral peaks from two methicillin resistant *Staphylococcus aureus* strains. The software automatically labels prominent peaks, and these were the peaks considered in the analyses. One strain is an USA100 strain (top), and the other strain is an USA300 strain (bottom). The USA100 strain has the class A *mec* gene complex with the PSM-mec peak near 2415 *m/z* (arrow), and the USA300 strain lacks the class A *mec* gene complex and the PSM-mec peak. The x-axis depicts mass-charge, and the y-axis represents percent intensity. The widths of the bars and the size of the x-axis labels were modified after screen capture to improve figure clarity, but the original screen captures are available (S2 Figure & S3 Figure).

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Table 1

Results of using the 2415 *m/z* peak to predict *mecA* carriage in 283 consecutive *S. aureus* blood culture isolates from unique patients[a]

When compared to *mecA* carriage determined by Verigene	Peak identified in *S. aureus* by Vitek MS	
	2415 ± 2.00 *m/z*	2415 ± 4.00 *m/z*
Sensitivity	0.37 (51/137) CI 0.29-0.46	0.39 (53/137) CI 0.30-0.47
Specificity	1.00 (146/146) CI 0.98-1.00	0.97 (142/146) CI 0.93-0.99
Positive predictive value	1.00 (51/51) CI 0.93-1.00	0.93 (53/57) CI (0.83-0.98)
Negative predictive value	0.63 (146/232) CI 0.56-0.69	0.63 (142/226) CI 0.56-0.69

[a] CI, 95% confidence interval.

Table 2

Results of using the 2415 *m/z* peak to predict *mecA* carriage in 243 consecutive *S. epidermidis* blood culture isolates from unique patients[a]

When compared to *mecA* carriage determined by Verigene	Peak identified in *S. epidermidis* by Vitek MS	
	2415 ± 2.00 *m/z*	2415 ± 4.00 *m/z*
Sensitivity	0.06 (10/180) CI 0.03-0.10	0.06 (10/180) CI 0.03-0.10
Specificity	0.98 (62/63) CI 0.91-1.00	0.97 (61/63) CI 0.89-1.00
Positive predictive value	0.91 (10/11) CI (0.59-1.00)	0.83 (10/12) CI (0.52-0.98)
Negative predictive value	0.27 (62/232) CI 0.21-0.33	0.26 (61/231) CI 0.21-0.33

[a] CI, 95% confidence interval.

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Analysis of the actual mass-charge ratios of isolates with a peak in the range of 2411-2419 *m/z* showed a roughly normal distribution of the mass-charge ratios (Figure 2 & S1 Dataset). In *S. aureus*, narrowing the PSM-mec peak range from 2415 ± 4.00 to 2415 ± 2.00 *m/z* eliminated the 4 "false positive" methicillin-susceptible *S. aureus* (MSSA) isolates, along with 2 MRSA isolates that were outliers (Figure 2). By narrowing the mass-charge ratio to 2415 ± 2.00 *m/z*, the specificity of predicting *mecA* carriage in *S. aureus* reached 100% (Table 1). Similarly, the specificity of this prediction for MRSE increased to 98% (Table 2).

When considering only MRSA isolates with a 2415 ± 2.00 *m/z* peak, the mean mass-charge ratio was 2414.55 (standard error of the mean of 0.091) with a standard deviation of 0.65. The peak in MRSE isolates had a mean mass-charge ratio of 2414.98 (standard error of the mean of 0.29) and a standard deviation of 0.90.

The set of twelve USA *S. aureus* strains contained three isolates with a detectable 2415 *m/z* peak using both Vitek MS (Figure 1) and Bruker Biotyper (Figure 3): USA100, USA200, and USA600. These strains carry SCC*mec* type II, which contains the class A *mec* gene complex that includes *psm-mec*. The nine other strains did not have the class A *mec* gene complex and did not have a 2415 *m/z* peak. When the twelve strains were analyzed using the Bruker MicroFlex, high intensity peaks were exclusively observed in USA100, USA200, and USA600. However, these peaks had a slightly lower mass-charge (2412 - 2414 *m/z* range) than the peaks detected using Vitek MS.

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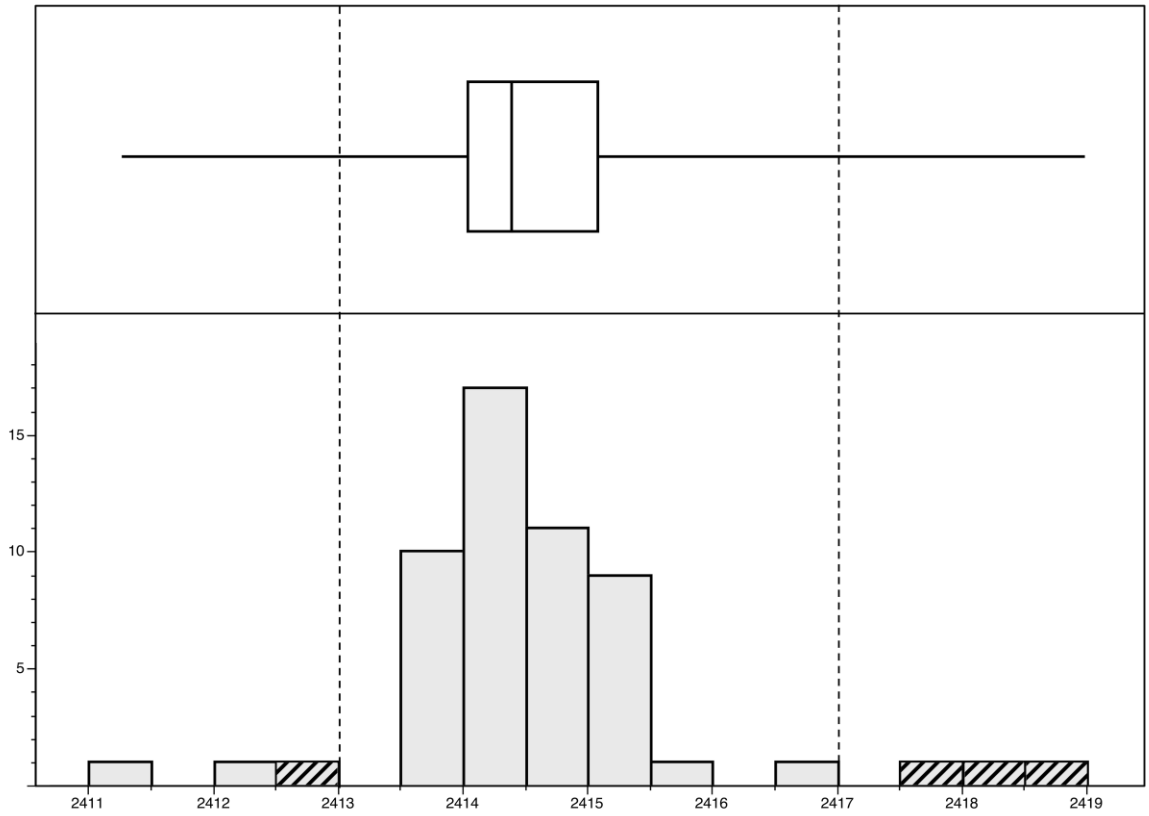

Figure 2 The distribution of the 2415 ± 4.00 *m/z* peaks identified in 57 blood culture isolates of *S. aureus* is depicted by a box-and-whisker plot (top) and a histogram (bottom). The box-and-whisker plot depicts the quartiles of peak distribution. Within the histogram, the four isolates lacking *mecA* are represented by hatched bars, and the 53 isolates of MRSA are represented by open bars. The vertical dotted lines represent a cut-off of 2415 ± 2.00 *m/z*, which improves the specificity of using the 2415 *m/z* peak to predict *mecA* carriage in *S. aureus*.

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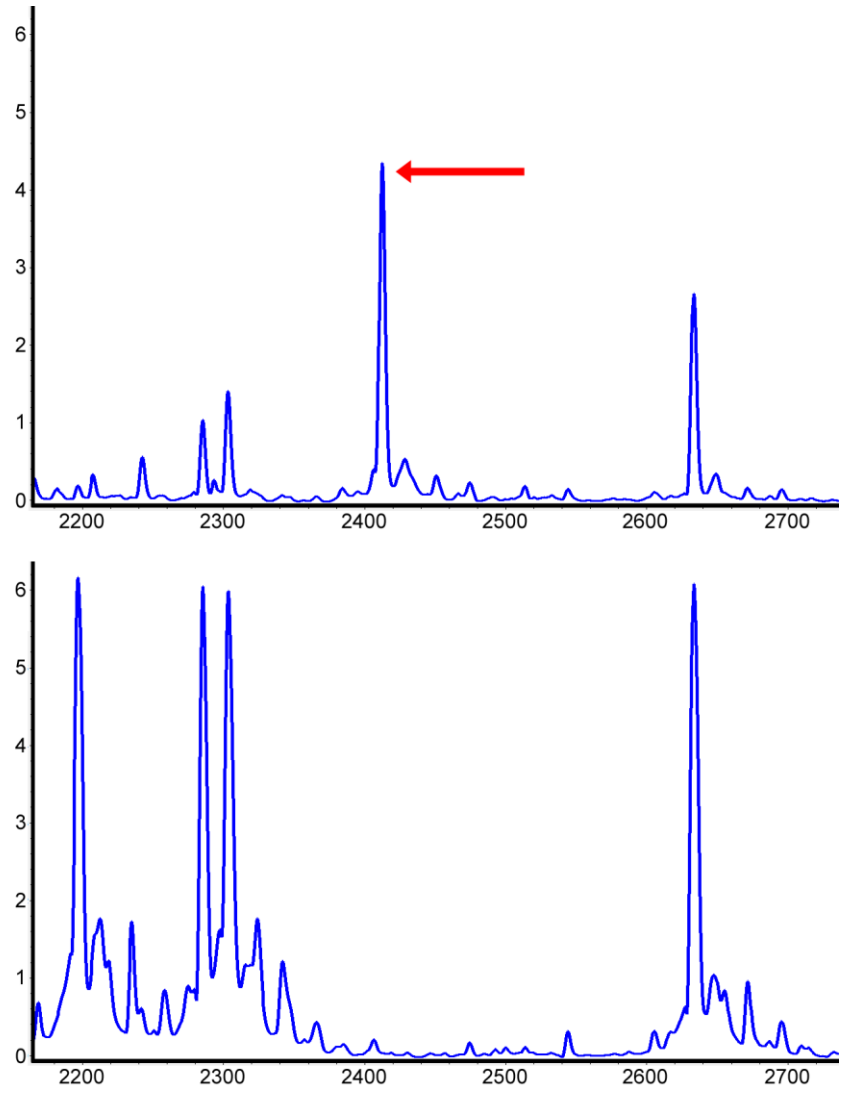

Figure 3

MALDI-TOF mass spectra from two methicillin resistant *Staphylococcus aureus* strains obtained using Bruker Biotyper are depicted. One strain is an USA100 strain (top), and the other strain is an USA300 strain (bottom). The USA100 strain has the class A *mec* gene complex with the PSM-mec peak near 2415 *m/z* (arrow), and the USA300 strain lacks the class A *mec* gene complex and PSM-mec. The x-axis depicts mass-charge, and the y-axis is arbitrary units x 10^4.

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210 This figure was modified from flexAnalysis screen captures. The original screen captures (S4 &

211 S5 Figures) and additional screen captures (Figures S6 & S7) are available.

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4. Discussion

Our findings demonstrate that the 2415 *m/z* peak predicts methicillin resistance with high specificity in *S. aureus* and *S. epidermidis*. This peak corresponds to the mass-charge ratio of the PSM-mec protein found in isolates with a class A *mec* gene complex, which has been previously described (Josten, et al., 2014). This is the first report of an attempt to demonstrate the feasibility of this method in a clinical laboratory setting.

In this study of consecutive blood isolates of *S. aureus*, 56% carried *mecA*. This carriage frequency is similar to the 57.1% average reported at five other medical centers across the United States (David, et al., 2014). Also, our finding that 37% of the MRSA isolates studied were positive for the 2415 ± 2.00 *m/z* peak is similar to the prevalence of the class A *mec* gene complex found in MRSA isolates at other centers (David, et al., 2014), although the *mec* class of these MRSA isolates was not evaluated in our study.

Our *post hoc* analysis demonstrated that narrowing the detection range from 2415 ± 4.00 to 2415 ± 2.00 *m/z* increased the specificity of this peak for predicting *mecA* carriage from 97% to 100% in *S. aureus*. This was a *post hoc* analysis, and the finding should be confirmed by studies in other laboratories using their own endemic strains and MALDI-TOF MS instrumentation.

Notably, the current study was performed using two Vitek MS systems with the IVD software package that has been cleared by the U.S. Food and Drug Administration, whereas a previous study was performed primarily using the Biflex II system (Bruker Daltonic, Bremen, Germany) (Josten, et al., 2014). Because the analysis can be performed using standard clinical software, it may facilitate the application of the analysis to routine clinical microbiology

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234 practice. Bruker has recently released software that would automate this analysis; the MBT
235 subtyping module is an RUO software solution designed specifically to detect the PSM-mec
236 peptide in *S. aureus* ("Bruker Introduces Important MALDI Biotyper Enhancements at ECCMID
237 2016: New Disposable MBT Biotargets 96, MBT Subtyping and Carbapenem Resistance Tests,"
238 2016). Using this software, if an isolate is identified as *S. aureus*, the software automatically
239 interrogates the mass spectrum for the PSM-mec peak and infers presumptive identification as
240 MRSA if the peak is present. Software solutions like this will foster routine clinical testing and
241 reporting of the PSM-mec peak.

242 Of the twelve well characterized USA *S. aureus* strains analyzed in our study, only those
243 with the class A *mec* gene complex yielded the 2415 *m/z* peak. This was true using both the
244 Vitek MS (Figure 1) and the Bruker Biotyper platform (Figure 3). However, PSM-mec's mass-
245 charge appeared slightly lower using the Bruker platform (~2413 *m/z*) than using the Vitek MS
246 systems, which was corroborated by another recent study (Budvytiene, Moon, Shi, & Banaei).
247 Until clinical microbiology has more experience with PSM-mec detection, each laboratory
248 should verify the mass-charge range which is most appropriate to use in each laboratory.

249 Data from this study are limited in that only blood isolates from our institution were
250 examined using our clinical instrumentation. The broader application of this study to other
251 specimen sources, other geographic regions, and other laboratory instrumentation has not been
252 evaluated. Verigene detection of *mecA* was used as the gold standard for determining methicillin
253 resistance in this study, and its concordance with other methods for detecting *mecA* has been
254 reported to be about 98% (Buchan, et al., 2013; Dodemont, De Mendonca, Nonhoff, Roisin, &
255 Denis, 2015; Mestas, Polanco, Felsenstein, & Dien Bard, 2014). It should be noted that the PSM-

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mec peptide is only detectable when cells are deposited directly on the target (Josten, et al., 2014). It is thought that ethanol extraction preparation washes away the PSM-mec peptide (Josten, et al., 2014; Szabados, Kaase, Anders, & Gatermann, 2012). It is not clear if PSM-mec detection is possible when using bacterial cells obtained directly from broth cultures (e.g. positive blood cultures). Because many MRSA and MRSE isolates carry a *mec* class other than class A, the absence of the 2415 *m/z* peak is not diagnostically informative and could be viewed as a limitation. The strength of analyzing spectra for the presence of the 2415 *m/z* peak is not in its sensitivity of detecting MRSA but in the specificity afforded when the peak is identified.

One isolate of *S. epidermidis* yielded a peak at 2415 ± 2.00 *m/z* even though no *mecA* gene was detected, and it tested susceptible to oxacillin using routine phenotypic methods. This isolate's original spectrum was reexamined, and the 2415 *m/z* peak that was identified and labeled by the Vitek MS Acquisition Station software had such low intensity that it was not detectable using subjective gestalt (Figures S8 & S9), which is in contrast to most other isolates' 2415 *m/z* peaks. This false positive result suggests that the specificity of the assay could be improved if the relative intensity of the 2415 *m/z* peak was required to reach a minimum threshold before being identified as positive. Alternately, a minimally acceptable ratio of 2415 *m/z* with a second peak that is consistently encountered in the taxon's mass spectrum could be used for objective evaluation of 2415 *m/z* peak intensity. Vitek MS Acquisition Station software is not designed for this type of analysis, so subjective interpretation or additional software features would be required to add an intensity criterion to the methodology used in this study.

One study examined the use of the 2415 *m/z* peak to identify MRSA with the class A *mec* gene complex, and they found that the sensitivity of the assay was significantly improved by

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278 excluding isolates with *agr* dysfunction (where *agr* dysfunction is identified by the absence of a
279 delta-toxin peak at 3007 *m/z*) (Josten, et al., 2014). Although this approach is appropriate when
280 attempting to use MALDI-TOF MS for strain typing, this approach is not necessary when only
281 using the 2415 *m/z* peak as a marker of methicillin resistance. We previously examined this
282 approach and concluded that when using the 2415 *m/z* peak as an early laboratory marker of
283 methicillin resistance, assessing *agr* function increased the complexity of the analysis without
284 improving the positive predictive value (Rhoads, Wang, Harrington, Procop, & Richter).

285 Although the 2415 *m/z* peak has been previously reported in coagulase negative
286 staphylococci (Josten, et al., 2014), this is the first study to describe the prevalence of the 2415
287 *m/z* peak in *S. epidermidis* clinical isolates. It is known that MRSE genetic resistance types are
288 diverse and varied (Cherifi, et al., 2013; Garza-Gonzalez, Morfin-Otero, Llaca-Diaz, &
289 Rodriguez-Noriega, 2010), and our finding that only 6% of MRSE isolates were positive for the
290 2415 *m/z* peak is not surprising. In spite of the low prevalence of the 2415 *m/z* peak in MRSE,
291 identifying the peak in an isolate of *S. epidermidis* still had a high positive predictive value for
292 identifying *mecA* carriage (Table 2).

293 Strengths of MALDI-TOF MS peak identification are simplicity and speed. An informal
294 time study determined that it took less than 30 seconds to use the Vitek MS Acquisition Station
295 software to retrieve a saved spectrum, identify the presence or absence of the 2415 *m/z* peak, and
296 record the results. Although manual analysis is rapid, easy, and inexpensive, ideally this analysis
297 would be performed by software in the future, which is similar to other MALDI-TOF MS
298 resistance marker analysis (Youn, et al., 2016).

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In the future, MALDI-TOF MS data will likely be used in the clinical laboratory for more than taxonomic identification of microorganisms. The finding of the 2415 *m/z* peak could be combined with more sophisticated MALDI-TOF MS software analyses to provide computer-interpreted staphylococcal strain lineage information (Camoez, et al., 2016; Josten, et al., 2014; Josten, et al., 2013; Wolters, et al., 2011), or the 2415 *m/z* characterization could be combined with other strain classification criteria to infer a strain type ("Use of an Inferred PFGE Algorithm, Emerging Infections Program / Active Bacterial Core (ABCs) Surveillance Invasive MRSA Project," 2009). For example, most MRSA strains with SCC*mec* Type II are classified as USA100 by inferred pulse field gel electrophoresis (PFGE) typing ("Active Bacterial Core Surveillance (ABC) Report Emerging Infections Program Network Methicillin-Resistant *Staphylococcus aureus*, 2013," 2015), and the PSM-mec peak in an isolate's mass spectrum could be used as a surrogate of SCCmec Type II detection.. Discriminating MALDI-TOF MS peak patterns could be used routinely by the clinical laboratory in partnership with infection prevention practitioners to monitor circulating bacteria strains (Spinali, et al., 2015; Youn, et al., 2016). Ideally, this surveillance would occur passively by continuous computerized interrogation of the mass spectra collected within the microbiology laboratory (Rhoads, Sintchenko, Rauch, & Pantanowitz, 2014; Youn, et al., 2016).

In summary, the absence of the 2415 *m/z* peak is not diagnostically informative, but its presence in an *S. aureus* or *S. epidermidis* clinical isolate is highly specific for predicting *mecA* carriage. Recognizing the presence of this 2415 *m/z* peak could expedite the identification of a subset of methicillin-resistant staphylococci (e.g. USA100 strains) without adding any reagent cost and only minimal labor.

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Supplementary material descriptions

S1 Dataset. Exact values of peaks in clinical isolates. Two sheets in an Excel (Microsoft, Redmond, CA) document contain results for *S. aureus* and *S. epidermidis* clinical isolates for peaks in the range of 2415 ± 4.00 *m/z*. The values were determined by Vitek MS Acquisition software as described in the methods section.

S2 Figure. Screen capture of the Vitek MS Acquisition software displaying results of USA100.

S3 Figure. Screen capture of the Vitek MS Acquisition software displaying results of USA300.

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S4 Figure. Screen capture of the Bruker flexAnalysis software displaying results of USA100.

S5 Figure. Screen capture of the Bruker flexAnalysis software displaying results of USA300.

S6 Figure. Screen capture of the Bruker flexAnalysis software displaying results of all 12 USA strains. USA100, USA200, and USA600 strains are depicted in red. Other strains are depicted in blue.

S7 Figure. Screen capture of the Bruker flexAnalysis software displaying zoomed in results of all 12 USA strains. USA100, USA200, and USA600 strains are depicted in red. Other strains are depicted in blue.

S8 Figure. Screen capture of the Vitek MS Acquisition software displaying results of the false positive *S. epidermidis* isolate. The 500 *m/z* window used in the study is depicted. The software automatically identified and labeled a peak at 2416.8 *m/z*, but this peak was not subjectively evident.

S9 Figure. Screen capture of the Vitek MS Acquisition software displaying results of the false positive *S. epidermidis* isolate. A zoomed in window of approximately 50 *m/z* is depicted.

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363 The software automatically identified and labeled a peak at 2416.8 *m/z*, but this peak was not

364 subjectively evident.

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References

Active Bacterial Core Surveillance (ABC) Report Emerging Infections Program Network Methicillin-Resistant *Staphylococcus aureus*, 2013 (2015). http://www.cdc.gov/abcs/reports-findings/survreports/mrsa13.pdf. updated 16 March 2015. accessed 13 May 2016.

Bruker Introduces Important MALDI Biotyper Enhancements at ECCMID 2016: New Disposable MBT Biotargets 96, MBT Subtyping and Carbapenem Resistance Tests (2016). http://ir.bruker.com/investors/press-releases/press-release-details/2016/Bruker-Introduces-Important-MALDI-Biotyper-Enhancements-at-ECCMID-2016-New-Disposable-MBT-Biotargets-96-MBT-Subtyping-and-Carbapenem-Resistance-Tests/default.aspx. updated 11 April 2016. accessed 23 June 2016.

Buchan, BW, Ginocchio, CC, Manii, R, Cavagnolo, R, Pancholi, P, Swyers, L, Thomson, RB, Jr., Anderson, C, Kaul, K, & Ledeboer, NA (2013) Multiplex identification of gram-positive bacteria and resistance determinants directly from positive blood culture broths: evaluation of an automated microarray-based nucleic acid test. *PLoS medicine, 10*: e1001478.

Budvytiene, I, Moon, HW, Shi, G, & Banaei, N Matrix-assisted laser desorption ionization time-of-flight mass spectrometry-based identification of methicillin-resistant *Staphylococcus aureus* through detection of a single peak. *(Poster presented on 19 June 2016 at ASM Microbe in Boston, MA).*

Camoez, M, Sierra, JM, Dominguez, MA, Ferrer-Navarro, M, Vila, J, & Roca, I (2016) Automated categorization of methicillin-resistant Staphylococcus aureus clinical isolates

DOI: http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.001

into different clonal complexes by MALDI-TOF mass spectrometry. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 22*: 161 e161-167.

Chatterjee, SS, Chen, L, Joo, HS, Cheung, GY, Kreiswirth, BN, & Otto, M (2011) Distribution and regulation of the mobile genetic element-encoded phenol-soluble modulin PSM-mec in methicillin-resistant Staphylococcus aureus. *PloS one, 6*: e28781.

Cherifi, S, Byl, B, Deplano, A, Nonhoff, C, Denis, O, & Hallin, M (2013) Comparative epidemiology of Staphylococcus epidermidis isolates from patients with catheter-related bacteremia and from healthy volunteers. *Journal of clinical microbiology, 51*: 1541-1547.

David, MZ, Daum, RS, Bayer, AS, Chambers, HF, Fowler, VG, Jr., Miller, LG, Ostrowsky, B, Baesa, A, Boyle-Vavra, S, Eells, SJ, Garcia-Houchins, S, Gialanella, P, Macias-Gil, R, Rude, TH, Ruffin, F, Sieth, JJ, Volinski, J, & Spellberg, B (2014) Staphylococcus aureus bacteremia at 5 US academic medical centers, 2008-2011: significant geographic variation in community-onset infections. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America, 59*: 798-807.

Diagnostic test evaluation calculator (2016). Version 16.2.1. https://www.medcalc.org/calc/diagnostic_test.php. updated 4 March 2016. accessed 16 March 2016.

Dodemont, M, De Mendonca, R, Nonhoff, C, Roisin, S, & Denis, O (2015) Evaluation of Verigene Gram-Positive Blood Culture Assay performance for bacteremic patients. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology, 34*: 473-477.

DOI: http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.001

411 Du, Z, Yang, R, Guo, Z, Song, Y, & Wang, J (2002) Identification of Staphylococcus aureus and
412 determination of its methicillin resistance by matrix-assisted laser desorption/ionization
413 time-of-flight mass spectrometry. *Analytical chemistry, 74*: 5487-5491.
414 Garza-Gonzalez, E, Morfin-Otero, R, Llaca-Diaz, JM, & Rodriguez-Noriega, E (2010)
415 Staphylococcal cassette chromosome mec (SCC mec) in methicillin-resistant coagulase-
416 negative staphylococci. A review and the experience in a tertiary-care setting.
417 *Epidemiology and infection, 138*: 645-654.
418 Josten, M, Dischinger, J, Szekat, C, Reif, M, Al-Sabti, N, Sahl, HG, Parcina, M, Bekeredjian-
419 Ding, I, & Bierbaum, G (2014) Identification of agr-positive methicillin-resistant
420 Staphylococcus aureus harbouring the class A mec complex by MALDI-TOF mass
421 spectrometry. *International journal of medical microbiology : IJMM, 304*: 1018-1023.
422 Josten, M, Reif, M, Szekat, C, Al-Sabti, N, Roemer, T, Sparbier, K, Kostrzewa, M, Rohde, H,
423 Sahl, HG, & Bierbaum, G (2013) Analysis of the matrix-assisted laser desorption
424 ionization-time of flight mass spectrum of Staphylococcus aureus identifies mutations
425 that allow differentiation of the main clonal lineages. *Journal of clinical microbiology,*
426 *51*: 1809-1817.
427 Kaito, C, Saito, Y, Ikuo, M, Omae, Y, Mao, H, Nagano, G, Fujiyuki, T, Numata, S, Han, X,
428 Obata, K, Hasegawa, S, Yamaguchi, H, Inokuchi, K, Ito, T, Hiramatsu, K, & Sekimizu,
429 K (2013) Mobile genetic element SCCmec-encoded psm-mec RNA suppresses
430 translation of agrA and attenuates MRSA virulence. *PLoS pathogens, 9*: e1003269.
431 Kaito, C, Saito, Y, Nagano, G, Ikuo, M, Omae, Y, Hanada, Y, Han, X, Kuwahara-Arai, K,
432 Hishinuma, T, Baba, T, Ito, T, Hiramatsu, K, & Sekimizu, K (2011) Transcription and

DOI: http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.001

translation products of the cytolysin gene psm-mec on the mobile genetic element SCCmec regulate Staphylococcus aureus virulence. *PLoS pathogens, 7*: e1001267.

Mestas, J, Polanco, CM, Felsenstein, S, & Dien Bard, J (2014) Performance of the Verigene Gram-positive blood culture assay for direct detection of Gram-positive organisms and resistance markers in a pediatric hospital. *Journal of clinical microbiology, 52*: 283-287.

Ostergaard, C, Hansen, SG, & Moller, JK (2015) Rapid first-line discrimination of methicillin resistant Staphylococcus aureus strains using MALDI-TOF MS. *International journal of medical microbiology : IJMM, 305*: 838-847.

Otto, M (2014a) Phenol-soluble modulins. *International journal of medical microbiology : IJMM, 304*: 164-169.

Otto, M (2014b) Staphylococcus aureus toxins. *Current opinion in microbiology, 17*: 32-37.

Patel, R (2015) MALDI-TOF MS for the diagnosis of infectious diseases. *Clinical chemistry, 61*: 100-111.

Queck, SY, Khan, BA, Wang, R, Bach, TH, Kretschmer, D, Chen, L, Kreiswirth, BN, Peschel, A, Deleo, FR, & Otto, M (2009) Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA. *PLoS pathogens, 5*: e1000533.

Rhoads, DD, Sintchenko, V, Rauch, CA, & Pantanowitz, L (2014) Clinical microbiology informatics. *Clinical microbiology reviews, 27*: 1025-1047.

Rhoads, DD, Wang, H, Harrington, SM, Procop, GW, & Richter, SS Evaluation of the 2415 *m/z* peak in the MALDI-TOF mass spectra of *Staphylococcus aureus* to infer methicillin resistance. *(Poster presented on 19 June 2016 at ASM Microbe in Boston, MA).*

DOI: http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.001

454 Spinali, S, van Belkum, A, Goering, RV, Girard, V, Welker, M, Van Nuenen, M, Pincus, DH,
455 Arsac, M, & Durand, G (2015) Microbial typing by matrix-assisted laser desorption
456 ionization-time of flight mass spectrometry: do we need guidance for data interpretation?
457 *Journal of clinical microbiology, 53*: 760-765.
458 Szabados, F, Kaase, M, Anders, A, & Gatermann, SG (2012) Identical MALDI TOF MS-derived
459 peak profiles in a pair of isogenic SCCmec-harboring and SCCmec-lacking strains of
460 Staphylococcus aureus. *The Journal of infection, 65*: 400-405.
461 Tansarli, GS, Karageorgopoulos, DE, Kapaskelis, A, & Falagas, ME (2013) Impact of
462 antimicrobial multidrug resistance on inpatient care cost: an evaluation of the evidence.
463 *Expert review of anti-infective therapy, 11*: 321-331.
464 Use of an Inferred PFGE Algorithm, Emerging Infections Program / Active Bacterial Core
465 (ABCs) Surveillance Invasive MRSA Project (2009).
466 http://www.cdc.gov/HAI/settings/lab/inferred-PFGE-algorithm.html. updated 2
467 December 2010. accessed 13 May 2016.
468 Wojewoda, CM, Sercia, L, Navas, M, Tuohy, M, Wilson, D, Hall, GS, Procop, GW, & Richter,
469 SS (2013) Evaluation of the Verigene Gram-positive blood culture nucleic acid test for
470 rapid detection of bacteria and resistance determinants. *Journal of clinical microbiology,*
471 *51*: 2072-2076.
472 Wolters, M, Rohde, H, Maier, T, Belmar-Campos, C, Franke, G, Scherpe, S, Aepfelbacher, M, &
473 Christner, M (2011) MALDI-TOF MS fingerprinting allows for discrimination of major
474 methicillin-resistant Staphylococcus aureus lineages. *International journal of medical*
475 *microbiology : IJMM, 301*: 64-68.

DOI: http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.001

476 Youn, JH, Drake, SK, Weingarten, RA, Frank, KM, Dekker, JP, & Lau, AF (2016) Clinical
477 Performance of a Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass
478 Spectrometry Method for Detection of Certain blaKPC-Containing Plasmids. *Journal of*
479 *clinical microbiology, 54*: 35-42.

480

481

DOI: http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.001