# The discriminatory power of MALDI-TOF mass spectrometry to differentiate between isogenic teicoplanin-susceptible and teicoplanin-resistant strains of methicillin-resistant *Staphylococcus aureus*

Paul A. Majcherczyk[1], Therese McKenna[2], Philippe Moreillon[1] & Pierre Vaudaux[3]

[1]Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; [2]Waters Corporation, Atlas Park, Manchester, UK; and [3]Service of Infectious Diseases, University Hospital of Geneva, Geneva, Switzerland

**Correspondence:** Paul Anthony Majcherczyk, Department of Fundamental Microbiology, University of Lausanne, 1015 Lausanne, Switzerland. Tel.: +41 793 038 971; fax +41 216 925 605; e-mail: Paul.Majcherczyk@unil.ch

Received 17 August 2005; revised 18 November 2005; accepted 18 November 2005.
First published online February 2006.

doi:10.1111/j.1574-6968.2005.00060.x

Editor: Jan-Ingmar Block

**Keywords**
*Staphylococcus aureus*; methicillin resistance; teicoplanin resistance; MALDI-TOF mass spectrometry; peptidoglycan muropeptide analysis; pulsed-field gel electrophoresis.

## Abstract

To explore the discriminatory power of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for detecting subtle differences in isogenic isolates, we tested isogenic strains of *Staphylococcus aureus* differing in their expression of resistance to methicillin or teicoplanin. More important changes in MALDI-TOF MS spectra were found with strains differing in methicillin than in teicoplanin resistance. In comparison, very minor or no changes were recorded in pulsed-field gel electrophoresis profiles or peptidoglycan muropeptide digest patterns of these strains, respectively. MALDI-TOF MS might be useful to detect subtle strain-specific differences in ionizable components released from bacterial surfaces and not from their peptidoglycan network.

## Introduction

The bacterial cell surface is a rich source of potential biomarkers that include cell receptors, adhesins, and antigenic determinants. Recent developments in a number of mass spectrometry techniques in particular matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) have provided opportunities to rapidly evaluate the surface of intact cells. MALDI-TOF MS allows direct analysis of surface components from bacterial colony suspensions. Sample preparation is simple and the analysis automated.

MALDI-TOF MS of intact microorganisms has been shown to produce characteristic mass spectral fingerprints of moieties desorbed from the cell surface (Claydon *et al.*, 1996; Holland *et al.*, 1996; Krishnamurthy *et al.*, 1996; Welham *et al.*, 1998; Bright *et al.*, 2002; Keys *et al.*, 2004), allowing this information to be used for rapid identification of Gram-negative and Gram-positive species and even differentiate between strains of the same species (Fenselau, 1994; Claydon *et al.*, 1996; Bernardo *et al.*, 2002; Du *et al.*, 2002; Walker *et al.*, 2002). Since many surface properties influence microbial physiology (e.g. electron transport, signal transduction), and virulence (e.g. toxin assemble, haemagglutins, ligands, binding receptors), it can also provide information on global modifications of such cell surface components (Shah *et al.*, 2002; Keys *et al.*, 2004). Moreover, MALDI-TOF MS has also been used to identify the impact of stress exposure on surface composition, e.g. in bifidobacteria exposed to bile salts (Marvin-Guy *et al.*, 2004).

In order to explore the discriminatory power of MALDI-TOF MS for detecting subtle differences in isogenic isolates, we used a panel of previously described isogenic strains of *Staphylococcus aureus* differing in their expression of antibiotic resistance (Vaudaux *et al.*, 2001, 2003; Renzoni *et al.*, 2004). Indeed emergence of multiresistant isolates represents a major threat for hospitalized patients. The prototype of such multiresistant hospital isolates is the methicillin-resistant *S. aureus* (MRSA), frequently associated with multiple additional resistance determinants (Waldvogel, 1999). The glycopeptide antibiotics vancomycin and teicoplanin are the most commonly used drugs to treat such

MRSA infections. Glycopeptides act by binding to the D-Ala–D-Ala termini of the nascent lipid II-linked murein precursor thereby inhibiting the polymerization of the bacterial wall glycan chains, and hence the cross-linking of the peptide moiety of the peptidoglycan. Teicoplanin-resistant isolates selected by glycopeptide therapy frequently exhibit a greater increase in minimal inhibitory concentrations (MICs) of teicoplanin than of vancomycin (Kaatz *et al.*, 1990). A similar observation was made after *in vitro* selection of organisms resistant to teicoplanin compared with those resistant to vancomycin (Kaatz *et al.*, 1990; Shlaes *et al.*, 1993; Mainardi *et al.*, 1995). Detailed characterization of clinical isolates and *in vitro*-selected glycopeptide-resistant subpopulations indicated frequent changes in cell wall thickening and metabolism (Daum *et al.*, 1992; Shlaes *et al.*, 1993; Mainardi *et al.*, 1995; Sieradzki & Tomasz, 1996, 1997, 1998, 1999; Hiramatsu *et al.*, 1997; Moreira *et al.*, 1997; Hanaki *et al.*, 1998; Hiramatsu, 1998; Sieradzki *et al.*, 1999; Cui *et al.*, 2000; Kuroda *et al.*, 2000; Boyle-Vavra *et al.*, 2001; Komatsuzawa *et al.*, 2002).

The aim of this report was to evaluate the sensitivity and specificity of MALDI-TOF MS for detecting potential alterations on the surface of the previously described isogenic *S. aureus* isolates differing in their expression of methicillin or teicoplanin resistance (Vaudaux *et al.*, 2001; Vaudaux *et al.*, 2003; Renzoni *et al.*, 2004). For comparison, the pulsed-field gel electrophoresis (PFGE) patterns and the peptidoglycan muropeptide patterns obtained from enzymatic digests of these isolates were also examined (Glauner, 1988; de Jonge *et al.*, 1993; Murchan *et al.*, 2003).

## Materials and methods

### Bacterial strains

The properties of MRSA teicoplanin-susceptible ($Tei^s$) parental strain MRGR3, its stable teicoplanin-resistant ($Tei^r$) derivative strain 14-4 and its teicoplanin-sensitive revertant, strain 14-4rev were previously described (Renzoni *et al.*, 2004). In addition, a spontaneous methicillin-susceptible revertant of MRGR3, strain Rev1 was also studied (Vaudaux *et al.*, 2003; Renzoni *et al.*, 2004). The strains were stored at $-70\,^{\circ}C$ in skimmed milk. They were cultured on Columbia blood agar (Oxoid, Basingstoke, UK) supplemented with 5% [volume in volume (v/v)] horse blood (TCS Microbiology, Bucks, UK) at $37\,^{\circ}C$. Their identity was confirmed by a real-time PCR method (Lausanne University Hospital, Lausanne, Switzerland) for the *femA* gene (to confirm that they were *Staphylococcus aureus*) and *mecA* gene (to confirm their resistance to methicillin). Primary sub-cultures were used for PFGE analysis, peptidoglycan muropeptide analysis and MALDI-TOF MS. MICs of teicoplanin for strain MRGR3, 14-4, 14-4rev are 1-2, 16 and $1\,\mu g\,mL^{-1}$, respectively, in cation adjusted Mueller-Hinton broth (Renzoni *et al.*, 2004). Except for the loss of the methicillin resistance element, strain Rev1 exhibits an antibiotic resistance pattern identical to its MRSA parent (Vaudaux *et al.*, 2003).

### MALDI-TOF MS of intact bacterial cells

Bacteria were cultured on Columbia blood agar supplemented with 5% (v/v) horse blood at $37\,^{\circ}C$. Single colonies grown for 24 h were removed from plates using a sterile loop and applied directly onto a 96-position MALDI sample target [Waters Corporation (Micromass Ltd), Manchester, UK]. The samples were allowed to air dry for 5 min before being overlaid with 1 μL of 5-chloro-2-mercaptobenzothiazole ($3\,\mu g\,mL^{-1}$) saturated solution in water, methanol, and acetonitrile, (1 : 1 : 1), 0.1% formic acid and 0.01 M 18-crown-6-ether (Keys *et al.*, 2004). The prepared target was air dried for 1 h prior to MALDI-TOF analysis in linear positive ion mode using the MALDI Linear Time of Flight Mass Spectrometer (Micromass) using the conditions described by Keys *et al.* (2004). Mass spectra were analysed in batches of 12 replicates from each strain and compared for reproducibility using the root mean square (RMS) value, obtained by comparing each replicate in turn with the average of the other 11 replicates. An RMS rejection value of 3.0 was used to identify outliers significant at the 0.1% level (Keys *et al.*, 2004). Average spectra were produced for inclusion in a personalised peak database using the MicrobeLynx™ software (Micromass). Data processing and comparison of representative spectra of each test strain were compared to those already included in the MicrobeLynx™ release database software as previously described in detail (Keys *et al.*, 2004).

### PFGE analysis

DNA was extracted from overnight cultures in brain heart infusion broth, digested with *Sma*I and electrophoresed as previously described (Murchan *et al.*, 2003). Gels were analysed by BIONUMERICS™ software (Applied Maths BVBA, Sint-Martens-Lantem, Belgium).

### Peptidoglycan muropeptide analysis

Insoluble peptidoglycan was purified from cultures grown to mid-log phase in brain heart infusion broth using a previously described standard procedure (de Jonge *et al.*, 1992; Majcherczyk *et al.*, 2003). After removal of teichoic acids by hydrofluoric acid insoluble peptidoglycan was digested with muramidase, yielding soluble muropeptides (de Jonge *et al.*, 1992). These were reduced with sodium borohydride, prior to separation by HPLC as previously described (de Jonge *et al.*, 1992).

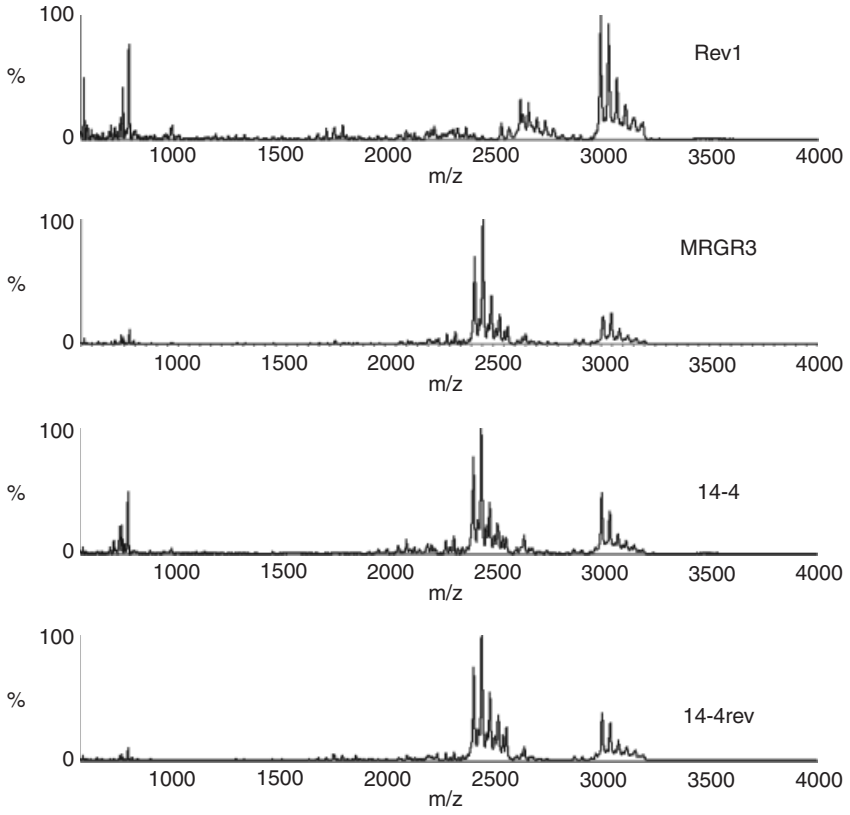


**Fig. 1.** Average spectra of the different strains tested. (a) MSSA strain Rev1; (b) methicillin-resistant *Staphylococcus aureus* (MRSA) teicoplanin-susceptible strain MRGR3; (c) its isogenic teicoplanin-resistant strain 14-4; (d) its isogenic teicoplanin-susceptible revertant 14-4rev.

## Results and discussion

### MALDI-TOF MS of whole bacterial cells

Initially, the test strains were analysed by MALDI-TOF MS and the resulting spectra of their ionisable cell surface components compared to spectra in the release database provided with MICROBELYNX$^{TM}$ software. MALDI-TOF MS of the 12 replicates for our test strains revealed highly reproducible (RMS $< 3$) ion spectra. The average spectra of our test strains closely matched spectra of reference *Staphylococcus aureus* strains in the MicrobeLynx$^{TM}$ software database, namely MSSA Rev1 with MSSA NCTC8532, MRSA Tei$^{s}$ MRGR3 with MRSA NCTC13131, MRSA Tei$^{r}$ 14-4 with MRSA NCTC13138, and MRSA Tei$^{s}$ 14-4rev with MRSA NCTC13131. Previous studies have reported that the spectrum was dependent on growth conditions of the strains before analysis and on the media used (Walker *et al.*, 2002). To minimise these effects all strains were cultured simultaneously on the same batch of medium. Reproducible results were obtained by using fresh cultures from different days.

Subsequently, spectra of the test strains were compared between themselves to see if differences could be observed between the isogenic test strains. Average spectra of strains Rev1, MRGR3, 14-4, and 14-4rev, are shown in Fig. 1. Visual inspection alone of the different spectra mainly shows the absence of a group of peaks around 2450 Da in Rev1 that is consistently present in the other strains tested. This suggests that the presence of the *mec*-element in the MRSA strains contributes to this group of peaks and is an agreement with previous studies that have shown major differences in spectra of MSRSA and MSSA and it served as a control in our study (Bernardo *et al.*, 2002; Du *et al.*, 2002; Walker *et al.*, 2002). Among the three MRSA strains differing in their teicoplanin susceptibilities, we noticed an increased

**Table 1.** Test strain spectra were compared with spectra in the MICROBELYNX$^{TM}$ personalized (*) and software release (**) databases

| | RMS values of database matched strain | | | | | | |
|---|---|---|---|---|---|---|---|
| Test strain | Rev1* | MRGR3* | 14-4* | 14-4rev* | MSSA 8532** | MRSA 13131** | MRSA 13138** |
| Rev1 | 0.58 | nr | nr | nr | 2.76 | nr | nr |
| MRGR3 | nr | 0.72 | 1.72 | 1 | nr | 3.13 | 3.31 |
| 14-4 | nr | 2.31 | 0.7 | 3.03 | nr | 6.88 | 4.2 |
| 14-4 rev | nr | 0.98 | 1.96 | 0.72 | nr | 2.34 | 2.85 |

Root mean square (RMS) values indicate the similarity between test strain spectra and database spectra. The lower the RMS, the greater the similarity. RMS $< 1$, very high similarity; RMS $> 1$ and $< 2$, high similarity; RMS $> 2$ and $< 3$, similar; RMS $> 3$ and $< 4$, low similarity; RMS $> 4$ and $< 7$, very low similarity; nr, not related.

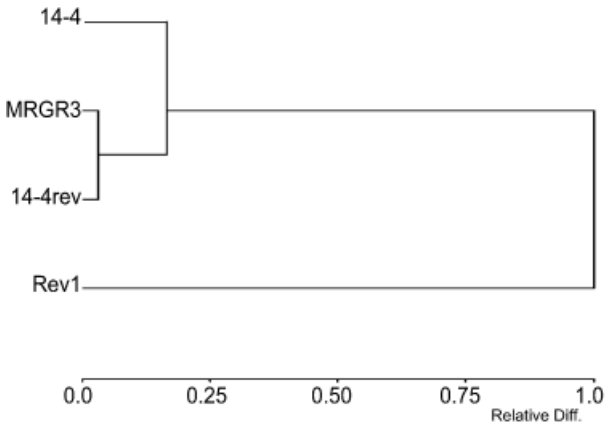


**Fig. 2.** Cluster analysis of mass spectra of the strains tested. Mass spectra were analysed with MICROBELYNX™ software. Relative Diff. represents relative difference, a relative scale normalized between 0 and 1. A difference of 0 indicates the clusters are exactly the same. A difference of 1 indicates that the clusters are the least similar clusters in the current data set.

intensity in some minor peaks at $< 2400$ Da in Tei$^r$ 14-4 compared to Tei$^s$ 14-rev and MRGR3.

A more accurate comparison of the test strains' spectra was achieved by using MICROBELYNX™ software. These results confirmed the visual impression that the MSSA Rev1 spectrum was significantly different (RMS $> 7$) from that of its isogenic MRSA counterparts (see Table 1). Among the latter strains, Tei$^s$ MRGR3 and 14-4rev were more closely related to each other (RMS $< 1.5$) than to Tei$^r$ 14-4 (RMS $> 1.5$). Figure 2 shows these results more clearly in the form of a cluster analysis. These results indicate that MALDI TOF MS can differentiate between isogenic strains of MRSA with different susceptibilities to teicoplanin. Moreover, the fact that MALDI-TOF MS analyses ionisable components from the cell surface strongly suggests the contribution of some unknown surface components to the strain-specific spectral differences.

## PFGE analysis

Figure 3 shows the PFGE patterns of the strains tested. MRGR3, 14-4rev and 14-4 yielded identical PFGE patterns except for a single-band difference in 14-4. The MSSA Rev1 had a three-band difference compared with the other MRSA strains tested. MALDI-TOF MS clustered (Fig. 2) the same strains together as the PFGE analysis (Fig. 3): MRSA and 14-4rev were more closely related to 14-4 than to Rev1.

## Muropeptide digest pattern analysis

To evaluate the potential contribution of cell wall peptidoglycan to MALDI-TOF MS-TOF MS strain-specific differences, we analysed solubilized muropeptides purified from each strain's peptidoglycan. As shown in Fig. 4, all tested

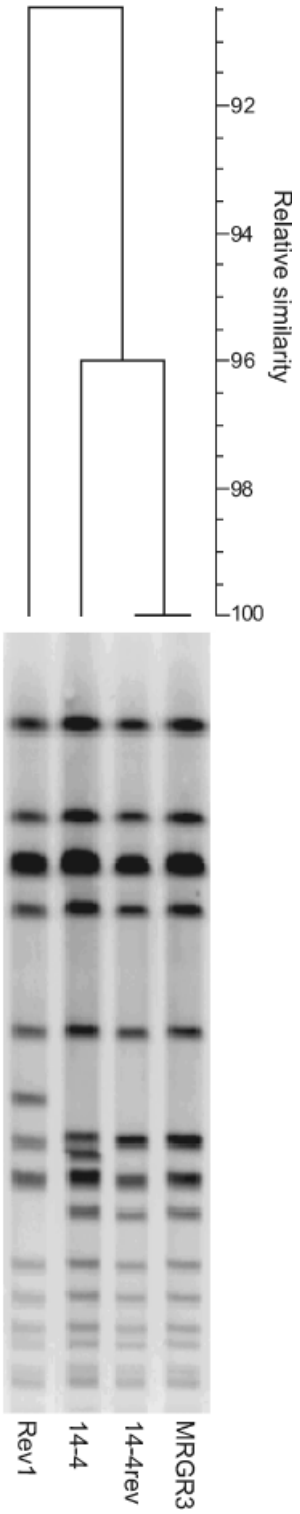


**Fig. 3.** Pulsed-field gel electrophoresis patterns of the strains tested. DNA was digested with *Sma*I and electrophoresed. Gels were analysed by the BIONUMERICS™ software. Relative similarity is a relative normalized scale between 100 and 0. A similarity of 100 indicates the clusters are exactly the same. A similarity of 0 indicates that the clusters are the least similar in the current data set.

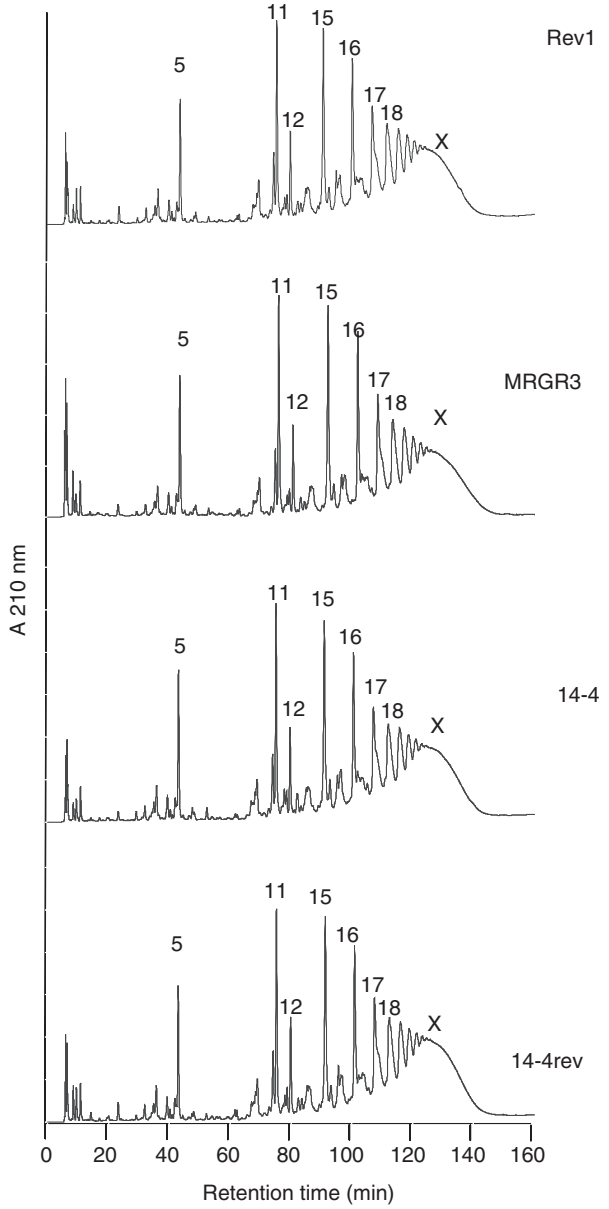


**Fig. 4.** Muropeptide fingerprints of MSSA Rev1, methicillin-resistant *Staphylococcus aureus* (MRSA) MRGR3, teicoplanin-resistant strain 14-4 and its teicoplanin-susceptible revertant, 14-4 rev. Muropeptides were prepared from Rev1, MRGR3 14-4 and 14-4 rev. Numbers identify major muropeptide components using previously described nomenclature – peaks 1–5 are monomeric muropeptides, 11 and 12 are dimeric muropeptides, 15, 16, 17 and 18 are tri-, tetra, penta- and hecta-meric muropetides respectively (de Jonge *et al*., 1992). The region X contains highly cross-linked muropeptides that were not resolved with the conditions used.

strains yielded identical muropeptide patterns, thus providing evidence that their peptidoglycan scaffold skeleton did not contribute to the cell surface alterations revealed by MALDI-TOF MS. These results support previous observations of de Jonge *et al*. who demonstrated that the muropeptide digest pattern of an MRSA strain was not significantly influenced by inactivation or deletion of the *mec* gene (de Jonge *et al*., 1992).

## Conclusions

Our observations that MALDI-TOF MS can easily differentiate between three isogenic MRSA derivatives and its spontaneously arising MSSA without showing any difference in their peptidoglycan muropeptide digest patterns bring additional support to previous observations reported by other laboratories that showed the power of MALDI-TOF MS for discriminating MRSA from MSSA strains of unrelated background (Edwards-Jones *et al*., 2000; Bernardo *et al*., 2002; Du *et al*., 2002; Walker *et al*., 2002). The present study brings further evidence that MALDI-TOF can successfully differentiate between isogenic strains considered identical by PFGE analysis. Our own data obtained on isogenic MRSA and MSSA isolates may facilitate identification of putative surface components altered by expression of antibiotic resistance. Further studies need to be performed on larger panels of isogenic antibiotic-resistant or susceptible isolates to evaluate the potential of MALDI-TOF MS for clinical microbiology applications.

## Acknowledgements

This work was supported by grants 3200B0-103793, 3200B0-108401 and 3200B0-103951 from the Swiss National Science Foundation. We thank Sonia Marra Majcherczyk and Diane Dare for excellent assistance.

## References

Bernardo K, Pakulat N, Macht M, Krut O, Seifert H, Fleer S, Hunger F & Kronke M (2002) Identification and discrimination of *Staphylococcus aureus* strains using matrix-assisted laser desorption/ionization-time of flight mass spectrometry. *Proteomics* **2**: 747–753.

Boyle-Vavra S, Labischinski H, Ebert CC, Ehlert K & Daum RS (2001) A spectrum of changes occurs in peptidoglycan composition of glycopeptide-intermediate clinical *Staphylococcus aureus* isolates. *Antimicrob Agents Chemother* **45**: 280–287.

Bright JJ, Claydon MA, Soufian M & Gordon DB (2002) Rapid typing of bacteria using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry and pattern recognition software. *J Microbiol Methods* **48**: 127–138.

Claydon MA, Davey SN, Edwards-Jones V & Gordon DB (1996) The rapid identification of intact microorganisms using mass spectrometry. *Nat Biotechnol* **14**: 1584–1586.

Cui L, Murakami H, Kuwahara-Arai K, Hanaki H & Hiramatsu K (2000) Contribution of a thickened cell wall and its glutamine nonamidated component to the vancomycin resistance

expressed by *Staphylococcus aureus* Mu50. *Antimicrob Agents Chemother* **44**: 2276–2285.

Daum RS, Gupta S, Sabbagh R & Milewski WM (1992) Characterization of *Staphylococcus aureus* isolates with decreased susceptibility to vancomycin and teicoplanin: isolation and purification of a constitutively produced protein associated with decreased susceptibility. *J Infect Dis* **166**: 1066–1072.

Du Z, Yang R, Guo Z, Song Y & Wang J (2002) Identification of *Staphylococcus aureus* and determination of its methicillin resistance by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Anal Chem* **74**: 5487–5491.

Edwards-Jones V, Claydon MA, Evason DJ, Walker J, Fox AJ & Gordon DB (2000) Rapid discrimination between methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* by intact cell mass spectrometry. *J Med Microbiol* **49**: 295–300.

Fenselau C (1994) *Mass Spectrometry for the Characterization of Microorganisms*, *Vol. 541* ACS Symposium Series, ACS, Washington.

Glauner B (1988) Separation and quantification of muropeptides with high-performance liquid chromatography. *Anal Biochem* **172**: 451–464.

Hanaki H, Labischinski H, Inaba Y, Kondo N, Murakami H & Hiramatsu K (1998) Increase in glutamine-non-amidated muropeptides in the peptidoglycan of vancomycin-resistant *Staphylococcus aureus* strain Mu50. *J Antimicrob Chemother* **42**: 315–320.

Hiramatsu K (1998) Glyopeptide resistance in staphylococci. *Drug Resistance Updates* **1**: 1350–1150.

Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchi Y & Kobayashi I (1997) Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet* **350**: 1670–1673.

Holland RD, Wilkes JG, Rafii F, Sutherland JB, Persons CC, Voorhees KJ & Lay JO Jr (1996) Rapid identification of intact whole bacteria based on spectral patterns using matrix-assisted laser desorption/ionization with time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom* **10**: 1227–1232.

de Jonge BL, Chang YS, Gage D & Tomasz A (1992) Peptidoglycan composition of a highly methicillin-resistant *Staphylococcus aureus* strain. The role of penicillin binding protein 2A. *J Biol Chem* **267**: 11248–11254.

de Jonge BL, Sidow T, Chang YS, Labischinski H, Berger-Bachi B, Gage DA & Tomasz A (1993) Altered muropeptide composition in *Staphylococcus aureus* strains with an inactivated femA locus. *J Bacteriol* **175**: 2779–2782.

Kaatz GW, Seo SM, Dorman NJ & Lerner SA (1990) Emergence of teicoplanin resistance during therapy of *Staphylococcus aureus* endocarditis. *J Infect Dis* **162**: 103–108.

Keys CJ, Dare DJ, Sutton H, Wells G, Lunt M, McKenna T, McDowall M & Shah HN (2004) Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterisation of bacteria implicated in human infectious diseases. *Infect Genet Evol* **4**: 221–242.

Komatsuzawa H, Ohta K, Yamada S, Ehlert K, Labischinski H, Kajimura J, Fujiwara T & Sugai M (2002) Increased glycan chain length distribution and decreased susceptibility to moenomycin in a vancomycin-resistant *Staphylococcus aureus* mutant. *Antimicrob Agents Chemother* **46**: 75–81.

Krishnamurthy T, Ross PL & Rajamani U (1996) Detection of pathogenic and non-pathogenic bacteria by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom* **10**: 883–888.

Kuroda M, Kuwahara-Arai K & Hiramatsu K (2000) Identification of the up- and down-regulated genes in vancomycin-resistant *Staphylococcus aureus* strains Mu3 and Mu50 by cDNA differential hybridization method. *Biochem Biophys Res Commun* **269**: 485–490.

Mainardi JL, Shlaes DM, Goering RV, Shlaes JH, Acar JF & Goldstein FW (1995) Decreased teicoplanin susceptibility of methicillin-resistant strains of *Staphylococcus aureus*. *J Infect Dis* **171**: 1646–1650.

Majcherczyk PA, Rubli E, Heumann D, Glauser MP & Moreillon P (2003) Teichoic acids are not required for *Streptococcus pneumoniae* and *Staphylococcus aureus* cell walls to trigger the release of tumor necrosis factor by peripheral blood monocytes. *Infect Immun* **71**: 3707–3713.

Marvin-Guy LF, Parche S, Wagniere S, Moulin J, Zink R, Kussmann M & Fay LB (2004) Rapid identification of stress-related fingerprint from whole bacterial cells of *Bifidobacterium lactis* using matrix assisted laser desorption/ionization mass spectrometry. *J Am Soc Mass Spectrom* **15**: 1222–1227.

Moreira B, Boyle-Vavra S, de Jonge BL & Daum RS (1997) Increased production of penicillin-binding protein 2, increased detection of other penicillin-binding proteins, and decreased coagulase activity associated with glycopeptide resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* **41**: 1788–1793.

Murchan S, Kaufmann ME, Deplano A, *et al.* (2003) Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *J Clin Microbiol* **41**: 1574–1585.

Renzoni A, Francois P, Li D, Kelley WL, Lew DP, Vaudaux P & Schrenzel J (2004) Modulation of fibronectin adhesins and other virulence factors in a teicoplanin-resistant derivative of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **48**: 2958–2965.

Shah HN, Keys CJ, Schmid O & Gharbia SE (2002) Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and proteomics: a new era in anaerobic microbiology. *Clin Infect Dis* **35**: S58–64.

Shlaes DM, Shlaes JH, Vincent S, Etter L, Fey PD & Goering RV (1993) Teicoplanin-resistant *Staphylococcus aureus* expresses a novel membrane protein and increases expression of

penicillin-binding protein 2 complex. *Antimicrob Agents Chemother* **37**: 2432–2437.

Sieradzki K & Tomasz A (1996) A highly vancomycin-resistant laboratory mutant of *Staphylococcus aureus*. *FEMS Microbiol Lett* **142**: 161–166.

Sieradzki K & Tomasz A (1997) Inhibition of cell wall turnover and autolysis by vancomycin in a highly vancomycin-resistant mutant of *Staphylococcus aureus*. *J Bacteriol* **179**: 2557–2566.

Sieradzki K & Tomasz A (1998) Suppression of glycopeptide resistance in a highly teicoplanin-resistant mutant of *Staphylococcus aureus* by transposon inactivation of genes involved in cell wall synthesis. *Microb Drug Resist* **4**: 159–168.

Sieradzki K & Tomasz A (1999) Gradual alterations in cell wall structure and metabolism in vancomycin-resistant mutants of *Staphylococcus aureus*. *J Bacteriol* **181**: 7566–7570.

Sieradzki K, Pinho MG & Tomasz A (1999) Inactivated pbp4 in highly glycopeptide-resistant laboratory mutants of *Staphylococcus aureus*. *J Biol Chem* **274**: 18942–18946.

Vaudaux P, Francois P, Berger-Bachi B & Lew DP (2001) In vivo emergence of subpopulations expressing teicoplanin or vancomycin resistance phenotypes in a glycopeptide-susceptible, methicillin-resistant strain of *Staphylococcus aureus*. *J Antimicrob Chemother* **47**: 163–170.

Vaudaux P, Francois P, Bisognano C, Li D, Lew DP & Schrenzel J (2003) Comparative efficacy of daptomycin and vancomycin in the therapy of experimental foreign body infection due to *Staphylococcus aureus*. *J Antimicrob Chemother* **52**: 89–95.

Waldvogel FA (1999) New resistance in *Staphylococcus aureus*. *N Engl J Med* **340**: 556–557.

Walker J, Fox AJ, Edwards-Jones V & Gordon DB (2002) Intact cell mass spectrometry (ICMS) used to type methicillin-resistant *Staphylococcus aureus*: media effects and inter-laboratory reproducibility. *J Microbiol Methods* **48**: 117–126.

Welham KJ, Domin MA, Scannell DE, Cohen E & Ashton DS (1998) The characterization of micro-organisms by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom* **12**: 176–180.