Identification and characterization of teicoplanin-intermediate
Staphylococcus aureus blood culture isolates in NE Scotland

Fiona M. MacKenzie1*, Paul Greig1, Donald Morrison2, Giles Edwards2 and Ian M. Gould1

1Medical Microbiology, Aberdeen Royal Infirmary, Forreshterhill, Aberdeen AB25 2ZN; 2Scottish MRSA Reference Laboratory, Microbiology Department, Glasgow Royal Infirmary, 84 Castle Street, Glasgow G4 0SF, UK

Received 30 November 2001; returned 7 March 2002; revised 20 March 2002; accepted 1 August 2002

The study objective was to screen both methicillin-resistant Staphylococcus aureus (MRSA) and methicillin-sensitive S. aureus (MSSA) isolates from blood cultures for reduced susceptibility to vancomycin and teicoplanin. A total of 72 MRSA and 143 MSSA isolates were screened on brain–heart infusion agar containing either 4 mg/L vancomycin or 8 mg/L teicoplanin, using an inoculum of ∼106 organisms. MICs were determined by Etest, broth microdilution and agar incorporation. Isolates were characterized by PFGE, mecA and nuc PCR, transmission electron microscopy (TEM) and analysis of cell proteins (proteomics). Based on British Society for Antimicrobial Chemotherapy (BSAC) breakpoints, seven MRSAs and seven MSSAs were teicoplanin resistant, with MICs of up to 16 and 24 mg/L respectively, but were vancomycin sensitive. Based on higher NCCLS breakpoints, five MRSAs and six MSSAs were teicoplanin intermediate, vancomycin sensitive. All the MRSAs belonged to the EMRSA-16 clone and subdivided into two groups. The MSSAs belonged to five different clones. TEM showed the resistant variants to have slightly thicker cell walls than sensitive variants. Most notably, the resistant variants possessed characteristic dark, granular material concentrated in the middle of the cells, believed to be chromosome. Proteomics showed the resistant variants to overexpress phosphoglycerate kinase. Both MRSA and MSSA with reduced teicoplanin susceptibility may remain vancomycin sensitive by NCCLS and BSAC criteria and it is important to screen clinical isolates of MRSA and MSSA for reduced susceptibility to both agents.

Introduction

The glycopeptides vancomycin and teicoplanin are of great importance in the treatment of patients with multiply-resistant Gram-positive infections. Indeed, there has been a dramatic increase in the use of glycopeptides, largely due to the treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections, with reports of a 20-fold increase in the decade 1981–1991.1,2 Recently however, there have been numerous, geographically diverse reports on the emergence of MRSA with reduced susceptibility to the glycopeptides and of failure of vancomycin treatment.3–13 Indeed, since the report of Hiramatsu et al.,3 vancomycin intermediate/resistant MRSA isolates have caused much alarm.

Since they were first described, there has been considerable confusion regarding the nomenclature of S. aureus with reduced susceptibility to the glycopeptides. Hiramatsu et al.3,4 first described an isolate of MRSA with a vancomycin MIC of 8 mg/L and called it a vancomycin-resistant S. aureus (VRSA). The first report from the USA however, called such isolates vancomycin-intermediate S. aureus (VISA) according to NCCLS MIC intermediate breakpoint values.5 The term ‘VISA’ was subsequently changed to the generic term ‘glycopeptide-intermediate S. aureus (GISA)’, on the basis that many of these isolates were also resistant to teicoplanin.14 There is however, no consensus and all of these terms are currently in use, although it has been suggested that the term ‘VISA’ is more meaningful to clinicians than the term ‘GISA’.15 To complicate nomenclature further, two resistance phenotypes, heterogeneous and homogeneous, have been recognized.3 To date there have only been 17 confirmed cases worldwide of homogeneous GISA. In contrast, there
has been a large number of reports of heterogeneous GISA. In this more common scenario, reduced glycopeptide susceptibility is expressed by a subpopulation (∼10⁶ cells), and the population is described as hetero-GISA (hGISA). Although their clinical relevance has been questioned, populations of hGISA can be associated with treatment failure and cause death. They are also thought to serve as precursors for homogeneous vancomycin resistance upon further glycopeptide exposure.¹⁶

Before the first reported GISA, the Centers for Disease Control and Prevention (CDC) in Atlanta recommended that all *S. aureus* and *Staphylococcus epidermidis* isolates be tested for reduced glycopeptide activity, but this has generally not occurred.¹⁷ Historically, the term ‘GISA’ has become attached to MRSA isolates with reduced susceptibility to vancomycin, and studies have generally looked for isolates meeting this narrow definition. The term ‘GISA’ however, is broader and, strictly speaking, incorporates both MSSA and MRSA isolates with intermediate resistance to either vancomycin or teicoplanin (VISAs or TISAs). The omission of MSSAs and teicoplanin from the majority of studies has left a deficiency in our knowledge that we believe is important to address. Part of the reason that less attention is paid to teicoplanin is that, until recently, it has only been in clinical use in Europe. Although it is still not licensed in the USA, it is now prescribed in Japan.

The fact that TISAs are of significance is demonstrated by the reported fatal consequences of failed teicoplanin therapy for severe infection caused by MSSA.¹⁸,¹⁹ Rapid identification of reduced susceptibility to teicoplanin may result in a more favourable outcome.²⁰,²¹ In these latter cases, the timely replacement of teicoplanin with vancomycin therapy led to full recovery of the patients. It has been suggested that vancomycin may generate isolates with reduced susceptibility to teicoplanin but not to vancomycin.²² In addition, it has been proposed that teicoplanin-resistant isolates may be one or two steps away from vancomycin resistance.¹⁶,¹⁸ and that teicoplanin is more likely to select glycopeptide resistance.¹⁶

As many hospitals in Europe use teicoplanin widely, especially in the treatment of MRSA infections, it is important to be able to recognize TISAs as well as VISAs. It therefore seems prudent to screen both MRSA and MSSA for reduced susceptibility to both vancomycin and teicoplanin. As disc diffusion testing, the most widely used susceptibility test system, cannot detect GISAs, it is essential to use specific screening and confirmatory tests for their detection.²² In this study, both MRSA and MSSA blood culture isolates from Aberdeen Royal Infirmary wards of high glycopeptide usage were screened retrospectively and putative GISAs were characterized.

### Materials and methods

**Bacterial strains**

A total of 72 MRSA and 143 MSSA blood culture isolates were screened for glycopeptide resistance. They were isolated at Aberdeen Royal Infirmary between August 1997 and August 1999 from six wards (ICU, haematology/oncology unit and renal dialysis unit) where glycopeptide usage was high. One glycopeptide-sensitive isolate (ATCC 29213), a homogeneous GISA (Mu50) and three heterogeneous GISA (Mu3, SMRL 99-3759 and SMRL 99-3700) were used as controls. Mu3 and Mu50 were kindly supplied by K. Hiramatsu, Japan.

**Screening**

Brain–heart infusion agar (BHIA) (Oxoid, Basingstoke, Hampshire, UK) plates were prepared containing either 4 mg/L vancomycin (Sigma-Aldrich Co. Ltd, Poole, Dorset, UK) or 8 mg/L teicoplanin (Aventis-Pharma).

An overnight culture of each isolate was diluted 1:10 and 10 µL (∼10⁶ organisms) plated on a quarter plate. The cell numbers were confirmed by a viable counting method.²³ Plates were examined after 24 and 48 h incubation at 37°C. Any colonies present were considered significant and tested further. All pre-screening cultures and post-screening colonies were stored at −70°C for further testing.

**Antimicrobial susceptibility testing**

Vancomycin and teicoplanin MICs were determined for all screen-positive isolates. Etest (Cambridge Diagnostics, Cambridge, UK) MICs were determined by standard methodology on Mueller–Hinton agar (MHA) with an inoculum equivalent to a 0.5 McFarland standard. This inoculum was made from a small sweep of colonies. Broth microdilution and agar incorporation MICs were determined using standard NCCLS methodology.²⁴ Methicillin MICs were determined for confirmed GISAs using the Etest method.

**Pulsed-field gel electrophoresis (PFGE)**

PFGE typing of *Sma*I (Gibco-BRL, UK) digested DNA was performed by a modification of a previously described method.²⁵ A colony was inoculated into BHI broth and incubated overnight at 37°C without agitation. The pellet from 0.4 mL of this culture was washed in 0.8 mL of NET buffer (10 mM Tris, 1 mM EDTA, 10 mM NaCl), resuspended in 0.25 mL of NET buffer and mixed with 200 U of achromopeptidase²⁶ (Sigma) and an equal volume of 2% SeaPlaque agarose (Flowgen, Ashby de la Zouch, UK) at 50°C. The cell/agarose suspension was loaded into block moulds (Bio-Rad Laboratories Ltd, UK) and allowed to solidify at 4°C. Cells were lysed by incubation at 50°C for 60 min in lysis buffer.
Teicoplanin-intermediate \textit{S. aureus} blood culture isolates

(6 mM Trizma base, 100 mM EDTA, 1 M NaCl, 0.5% Brij 58, 0.2% sodium deoxycholate, 0.5% lauroyl sarcosine). The blocks were washed three times for 10 min each at room temperature in TE buffer (10 mM Trizma base, 1 mM EDTA). One-quarter of each agarose block was digested with 30 U of \textit{Smal} for 3 h, according to the manufacturer’s instructions, and loaded into the wells of a 1% PFGE certified agarose gel (Bio-Rad Laboratories Ltd). Electrophoresis was performed in 0.5 × TBE buffer (44.5 mM Trizma base, 44.5 mM boric acid, 1 mM EDTA) (Biowhittaker, Wokingham, UK) by the contour clamped homogeneous electric field method with a CHEF Mapper system (Bio-Rad Laboratories Ltd). The fragments were separated with a linear ramped pulse time of 6.8–63.8 s over a period of 23 h at 14°C and the gels were stained with 1 µg/mL ethidium bromide (Sigma) solution for 30 min, visualized under UV and photographed.

\textit{mec}A and \textit{nuc} gene detection

In addition to \textit{mec}A, rRNA and 16S rRNA, primers described by Bignardi \textit{et al.},\textsuperscript{27} the \textit{S. aureus} species-specific \textit{nuc} gene primers\textsuperscript{28} were incorporated into a multiplex PCR assay as described previously.\textsuperscript{26,27,29}

Thin-section transmission electron microscopy (TEM)

Thin-section TEM was carried out based on the methods described previously\textsuperscript{30–32} on two pairs of pre- and post-screening isolates: one MSSA pair and one MRSA pair. The isolates were grown in 100 mL of tryptic soy broth for 48 h at 37°C; the pre-screening isolates were grown in the absence of antibiotic, whereas the post-screening isolates were grown in the presence of 8 mg/L teicoplanin. The cultures were centrifuged at 3000g for 15 min and the pellet was then fixed in a 2% paraformaldehyde/2.5% glutaraldehyde solution. The cells were washed in 0.1 M phosphate buffer, resuspended in 1% osmium tetroxide, washed further in distilled water and finally resuspended in 2% agarose. Small blocks of agarose containing suspended bacteria were then dehydrated through a graded series of ethanol solutions and embedded in araldite. Thin sections were cut using a diamond knife on a Reichert Ultracut E ultramicrotome, stained with uranyl acetate and lead citrate, and finally examined using a Phillips EM301 transmission electron microscope.

Proteomics (analysis of cell proteins)

Total cell protein analysis (proteomics) was performed according to standard methods\textsuperscript{33,34} on the same isolates as were visualized by TEM. Pre-screening isolates were grown overnight on Mueller–Hinton agar, and post-screening isolates were grown on Mueller–Hinton agar with 8 mg/L teicoplanin. The cells were harvested, suspended in lysis buffer with added lysostaphin and lysozyme, and sonicated.\textsuperscript{30} Bacterial proteins were analysed using a small-format two-dimensional gel system. The first dimension separated the proteins using a Multiphor II IEF kit (Amersham Pharmacia Biotech) and the second dimension separated the proteins by molecular weight, using polyacrylamide electrophoresis. The gels were finally stained using Coomassie Blue R250 (BDH, Poole, UK) and compared. Protein spots of interest were either missing in one gel of a pair or were differentially expressed within a pair of gels. Spots of interest were excised from the gel, digested with trypsin and spectra obtained using a mass spectrometer.\textsuperscript{35} Resultant profiles were then compared with protein sequence databases to identify the spots of interest.

Results

Screening

Twenty-one of the 72 MRSAs (29.2%) and seven of the 143 MSSAs (4.9%) grew on the screening agar. MICs were then determined for these colonies. Notably, the seven original cultures of MSSA detailed in Table 1 grew between one and seven colonies on the teicoplanin-screening plates only. Of the seven original MRSA cultures (Table 1), four grew only on teicoplanin-screening plates, two grew only on vancomycin-screening plates and one grew on both. Again, all of the MRSAs expressed heterogeneous teicoplanin resistance, with between 1 and >30 colonies observed on the screening plates.\textsuperscript{3}

MICs

Vancomycin and teicoplanin MICs were determined for post-screening, resistant subpopulations by three standard methods and interpreted according to both NCCLS and British Society for Antimicrobial Chemotherapy (BSAC) breakpoints (Table 2). Based on BSAC teicoplanin breakpoints, seven MSSAs and seven MRSAs were identified as teicoplanin resistant (Table 1). The 14 isolates were classified as teicoplanin resistant by at least one of the three MIC methods. According to the higher NCCLS breakpoints, however, only 11 isolates were classified as teicoplanin resistant by at least one of the three methods used. It should be noted that although NCCLS criteria state that an MIC ≤ 8 mg/L is classified as susceptible and 16 mg/L is classified as intermediate, Etest MIC values exist that fall between these values. This is because, unlike traditional methods, the Etest methods can determine MIC values that do not fall within doubling dilution ranges. Etest MIC values of 12 mg/L have therefore been classified as intermediate by NCCLS breakpoints.

All 14 isolates were sensitive to vancomycin according to both BSAC and NCCLS criteria (Table 2). It is notable however, that the MRSAs were consistently more sensitive to vancomycin than the MSSA isolates; all but one had broth MIC values of 4 mg/L and could be classified as ‘borderline
F. M. MacKenzie et al.

Table 1. Glycopeptide MIC values for post-screening cultures

<table>
<thead>
<tr>
<th>Strain ID</th>
<th>Clone</th>
<th>Pre-screen/post-screen methicillin MICs (mg/L)</th>
<th>Vancomycin MICs (mg/L)</th>
<th>Teicoplanin MICs (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>broth</td>
<td>agar</td>
<td>Etest</td>
</tr>
<tr>
<td>MSSA 1</td>
<td>SMSSA 111i</td>
<td>0.75/1.5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>MSSA 2</td>
<td>Sporadic</td>
<td>1/3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>MSSA 3</td>
<td>SMSSA 136</td>
<td>1/3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>MSSA 4</td>
<td>SMSSA 127i</td>
<td>0.75/1.5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>MSSA 5</td>
<td>SMSSA 127ii</td>
<td>1/1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>MSSA 6</td>
<td>SMSSA 111ii</td>
<td>0.75/2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>MSSA 7</td>
<td>SMSSA 105</td>
<td>1/3</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>MRSA 1</td>
<td>EMRSA 16a</td>
<td>&gt;256</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>MRSA 2</td>
<td>EMRSA 16a</td>
<td>&gt;256</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>MRSA 3</td>
<td>EMRSA 16a</td>
<td>&gt;256</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>MRSA 4</td>
<td>EMRSA 16d</td>
<td>&gt;256</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MRSA 5</td>
<td>EMRSA 16a</td>
<td>&gt;256</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>MRSA 6</td>
<td>EMRSA 16d</td>
<td>&gt;256</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>MRSA 7</td>
<td>EMRSA 16d</td>
<td>&gt;256</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

For each isolate the highest MIC, by any of the three methods, has been interpreted according to both NCCLS and BSAC breakpoints. The MIC values that fall into the intermediate or resistant categories by either NCCLS or BSAC breakpoints have been highlighted with an asterisk (*).

*Methicillin Etest MICs were determined for the original, pre-screening population and for the resistant subpopulation tested post-screening.
SMSSA is the term used by the Scottish Reference Laboratory to name Scottish MSSA types.

Table 2. Glycopeptide breakpoint values

<table>
<thead>
<tr>
<th>Glycopeptide</th>
<th>Susceptible</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>NCCLS ≤4 mg/L</td>
<td>8–16 mg/L</td>
<td>≥32 mg/L</td>
</tr>
<tr>
<td></td>
<td>BSAC ≤4 mg/L</td>
<td>–</td>
<td>≥8 mg/L</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>NCCLS ≤8 mg/L</td>
<td>16 mg/L</td>
<td>≥32 mg/L</td>
</tr>
<tr>
<td></td>
<td>BSAC ≤4 mg/L</td>
<td>–</td>
<td>≥8 mg/L</td>
</tr>
</tbody>
</table>

Discussion

In an attempt to pre-empt the emergence and spread of glycopeptide resistance, it was recommended that all S. aureus
isolates should be tested for reduced susceptibility to the glycopeptides. Despite the fact that clinically significant cases of MSSAs with reduced teicoplanin susceptibility have been described for over 10 years, the topic of glycopeptide resistance in *S. aureus* has only enjoyed real prominence since Hiramatsu *et al.* first described VRSA in 1997. Since then, studies have focused on MRSA isolates at the expense of MSSA isolates, and on vancomycin at the expense of teicoplanin. This is because most of the GISA work to date has been carried out in countries such as Japan and the USA, where teicoplanin has either been introduced recently or has not been licensed for use in clinical medicine. A more worrying trend is the fact that prevalence work in European countries, where teicoplanin is widely used to treat MRSA infections, continues to focus on vancomycin resistance and not teicoplanin resistance. The fact that the vast majority of VISA studies have focused on MRSA is surprising in that MSSA is still the more frequent pathogen in most centres. We believe that it is as important to detect reduced susceptibility to glycopeptides in MSSAs as in MRSA.

In countries such as the UK, treatment failure is as likely to be caused by TISAs as by VISAs. It is important to detect TISAs as early as possible in order to change to more appropriate antibiotic therapy and to instigate appropriate and rigorous infection control measures.

Despite the increasing profile of glycopeptide resistance, considerable confusion still remains over nomenclature and appropriate screening and confirmatory methods. The generic term ‘GISA’ was introduced because ‘most’ confirmed cases at that time were described as intermediate to both...
vancomycin and teicoplanin. On the basis of the current study and several other reports that described \textit{S. aureus} with reduced susceptibility to teicoplanin but not to vancomycin, we believe that the introduction of a new term ‘TISA’ (teicoplanin-intermediate \textit{S. aureus}) is appropriate. The distinction offered by this term, which includes both MRSA and MSSA, and the retention of the terms GISA and/or VISA for isolates with resistance to vancomycin, would be an advantage in the study of this phenomenon and would also be useful to clinicians.

A major methodological problem is that low-level glycopeptide resistance is not readily detected by disc diffusion testing and specific screening and confirmatory tests must be used. Although population analysis studies are considered the gold standard in GISA detection, they are not easily performed in the routine clinical laboratory. Proposed screening methods include the use of gradient plates, the \beta-lactam/glycopeptide antagonism test, the ‘macro’ Etest and the breakpoint agar method, which has been used in the majority of studies. Variations in parameters such as medium and vancomycin concentration alter the specificity and sensitivity of the agar screening test, although the consensus appears to be use of brain–heart infusion agar with a concentration of antibiotic on or around the sensitive breakpoint, use of a dense inoculum and 48 h incubation.

Various methods of confirming VISAs have been proposed. The CDC recommend that isolates: (1) should grow on BHI agar containing 6 mg/L vancomycin; (2) should have a vancomycin Etest \(\geq\) 6 mg/L; and (3) should have a vancomycin broth microdilution MIC of 8–16 mg/L, which are the NCCLS intermediate breakpoint values. There are currently no guidelines for confirming TISAs. Following NCCLS teicoplanin breakpoint values and the data from this study, it is suggested that TISA could be confirmed with: (1) growth on MHA containing 8 mg/L teicoplanin; (2) teicoplanin Etest MIC \(\geq\) 12 mg/L; and (3) broth microdilution teicoplanin MIC of 16 mg/L. Interestingly, Walsh \textit{et al.} proposed that a teicoplanin Etest MIC \(\geq\) 12 mg/L is predictive of hVISA. NCCLS is the largest organization involved in setting standards in susceptibility testing, and its agar disc diffusion method is the most widely adopted susceptibility test method. We must, however, acknowledge the fact that the BSAC has recommended ‘national’ breakpoints, which differ from those of the NCCLS. The BSAC teicoplanin breakpoint of 4 mg/L is, however, in agreement with the breakpoint set in both France and Sweden. BSAC glycopeptide breakpoint values are lower than NCCLS breakpoints and have no intermediate category (Table 2). Their appropriateness in the study of GISAs is questionable and they should perhaps be revisited. The use of lower breakpoints has been considered by other investigators and it has been proposed that isolates with a vancomycin MIC \(\geq\) 4 mg/L or a teicoplanin MIC \(\geq\) 8 mg/L might be considered to have reduced glycopeptide susceptibilities. Isolates with these slightly lower MICs have also been described as ‘borderline GISAs’.

Using BSAC interpretive criteria, a total of 9.7\% (seven) of the MRSA tested and 4.9\% (seven) of the MSSA were found to be hGRSAs, based on teicoplanin MICs. If only the more rigorous NCCLS breakpoints were applied, this would have excluded one of the MSSAs and two of the MRSA. These figures may seem unusually high when compared with a similar study carried out to detect reduced glycopeptide susceptibility in \textit{S. aureus} in England and Wales, which found no such isolates. However, the latter study used vancomycin not teicoplanin and was designed to detect only homogeneously resistant GISAs and not hGISAs. It should also be borne in mind that only \textit{S. aureus} blood culture isolates from patients in wards of high glycopeptide use were tested in the current study. These were thought to be the most likely areas in which to find GISAs, as their emergence elsewhere has been associated with prior teicoplanin therapy. All of the cells that displayed reduced teicoplanin susceptibility represented a subpopulation of the original, pre-screening culture. Of the original \(10^6\) cells applied to each screening plate, between one and 30 colonies grew in the presence of the glycopeptides. These were called hGISAs as defined by Hiramatsu \textit{et al.}, who stated that this definition should be applied to subpopulations showing reduced glycopeptide susceptibility at a frequency of \(10^6\) or higher.

In order to investigate the epidemiology of the hTISAs and to verify that the resistant subpopulations were identical to the original, pre-screening cultures, PFGE was carried out. It has been suggested that there is a specific \textit{S. aureus} clone which has a greater potential to generate glycopeptide resistance than other clones. Indeed the Japanese VISAs, five examples of VISA from America and eight of ‘borderline VISAs’ with vancomycin MICs of 4 mg/L all belonged to the same clone. It is one of the most common \textit{S. aureus} clones in Japan and has been reported worldwide under various names: clontype II-A, Paediatric clone, Belgian type 3, and in Scotland it is known as Type 105. Both MRSA and MSSA variants of Type 105 have been identified by the Scottish MRSA Reference Laboratory. One of the TISAs in this study belonged to the ‘VISA’ lineage. However, it is apparent from this study, where four further TISA clones were identified, that the potential to generate isolates with reduced susceptibility to the glycopeptides is not restricted to a single \textit{S. aureus} clone. It is particularly worrying that clones which have a remarkable potential for spreading nationally and internationally, the so-called ‘super’ epidemic clones, have this resistance. This is true for EMRSA-16, the second most common UK epidemic MRSA clone reported in this study. In addition, several other ‘super’ epidemic clones have been reported with this resistance, including EMRSA-15 (the most common UK MRSA clone), the Iberian clone and the Brazilian clone. A recent report of TISA in England designated the clone as...
EMRSA-17. With these clones, the potential for dissemination of VISA and TISA clones is very real, and prevention and control guidelines have been published.

Further characterization of some of our isolates was carried out by TEM and total protein analysis. By TEM, the sensitive populations showed cells of varying size and cell wall thickness. In keeping with the findings of other investigators, the resistant subpopulations were more consistent, with thicker cell walls. Possible reasons for the thickened cell walls are that they prevent the glycopeptides from accessing their targets, and glycopeptide exposure may produce an accumulation of peptidoglycan-soluble precursors that result in cell wall thickening. The TEM images of the S. aureus cells in this study were notable in that the cells exhibited dark, concentric, granular material, believed to be chromosome— which has not been described previously. Staining irregularities were ruled out, and the specific nature of this material is uncertain.

In this study, the total cell protein analysis found that the tested hGISA subpopulations overexpressed phosphoglycerate kinase (PGK) in relation to the related, pre-screening cultures. PGK is an enzyme involved in the glycolytic pathway. In particular, it is involved in phase II of glycolysis, in the most important step of substrate-level phosphorylation, that is, the conversion of 1,3-diphosphoglycerate (PGA) into 3PGA, yielding ATP molecules. The reason for the excess PGK in the resistant subpopulation can only be speculated. It is interesting to note that bacterial spores are associated with high levels of energy stored as phosphoglyceric acid. Spores have a very thick cortex, made up of loosely cross-linked peptidoglycan. The thicker cell wall of hGISAs is also associated with extra peptidoglycan and may be analogous to a ‘pre-spore’ stage. When germination of a spore takes place, it occurs rapidly and is associated with hydrolysis of peptidoglycan and the rapid release of energy due to substrate-level phosphorylation involving phosphoglyceric acid and the enzyme PGK.

Although each pre- and post-screening pair of isolates was found to be indistinguishable by PFGE, significant differences were seen in relation to the methicillin Etest MICs established for the MSSAs (P = 0.005792). Although the MSSAs were all mecA PCR negative and were therefore not classified as pre-MRSA, the glycopeptide-resistant subpopulation had significantly higher methicillin MICs than the original pre-screening populations (Table 1). Two of the MSSAs had methicillin MICs of 3 mg/L and were classified as borderline resistant. It has been recorded that GISA strains of Staphylococcus aureus with reduced susceptibility to vancomycin—United States. Morbidity and Mortality Weekly Report 46, 756–765.

In summary, we have introduced the new term ‘TISA’ to identify an important resistance phenotype. In doing so, we have identified seven MRSA and seven MSSA that are hGISAs, based on teicoplanin MICs ranging from 8 to 24 mg/L. Generally, the MSSAs had higher vancomycin MICs than the MRSA, which may be due to PBP-4 alterations associated with thick cell walls. The hGISAs overexpressed PGK, which, again, may be associated with thick cell walls. There is much uncertainty relating to the clinical importance of hGISAs, but we agree with other investigators that as much information as possible should be assimilated on these isolates until their role in therapeutic failure can be clarified.

Acknowledgements

We are grateful to Mrs Debbie Marshall for help with the electron microscopy and to Dr Phil Cash and Dr Laura Lawrie for help with the proteomics.

This work was presented in part at the following conferences: 3rd European Congress of Chemotherapy, Madrid, Spain, 7–10 May 2000. Abstract T130; 11th European Congress of Clinical Microbiology and Infectious Diseases, Istanbul, Turkey, 1–4 May 2001, Abstract 557.

References


Teicoplanin-intermediate S. aureus blood culture isolates


