Staphylococcus aureus with reduced glycopeptide susceptibility in Liverpool, UK

Andrew Kirby1*, Robert Graham2, Nicola J. Williams3, Mandy Wootton4, Caroline M. Broughton1, Mishaal Alanazi1, James Anson2, Timothy J. Neal2 and Christopher M. Parry1

1School of Infection and Host Defence, Duncan Building, University of Liverpool, Daulby Street, Liverpool L69 3GA, UK; 2Department of Medical Microbiology, Royal Liverpool and Broadgreen University Hospital Trust, Prescot Street, Liverpool L7 8XP, UK; 3School of Veterinary Science, National Centre for Zoonoses Research, University of Liverpool, Leahurst, Chester High Road, Neston, Wirral CH64 7TE, UK; 4Specialist Antimicrobial Chemotherapy Unit, NPHS Cardiff, University Hospital of Wales, Heath Park, Cardiff CF14 4XW, UK

*Corresponding author. Tel: +44-151-706-4396/4410; Fax: +44-151-706-5805; E-mail: amk@liv.ac.uk

Received 3 January 2010; accepted 5 January 2010

Objectives: To investigate if colonization with heterogeneous glycopeptide-intermediate Staphylococcus aureus (hGISA) is associated with hGISA bacteraemia.

Methods: Isolates of methicillin-resistant S. aureus (MRSA) from blood cultures and from swabs to detect MRSA colonization were screened for reduced susceptibility to glycopeptides by an agar incorporation method. Isolates detected by this screen were tested for glycopeptide resistance by MacroEtest, standard MIC Etest methods and population analysis profile–AUC (PAP–AUC) analysis. S. aureus isolates with and without reduced glycopeptide susceptibility were characterized by PFGE and spa typing.

Results: MRSA isolates with reduced susceptibility to glycopeptides, as identified by the MacroEtest method, were detected in the colonization screens of 86 of 2550 MRSA-positive patients. The isolates were confirmed by Etest MIC and PAP–AUC analysis as hGISA. A total of 82/86 of the hGISA colonizing isolates were EMRSA-16 by PFGE; the remainder were EMRSA-15. Bacteraemia with hGISA was identified in five patients during the study period; two isolates were EMRSA-16 and three were EMRSA-15. hGISA colonization could not be linked to hGISA bacteraemia and hGISA bacteraemia could not be linked to hGISA colonization. Four of the five hGISA bacteraemias developed following teicoplanin therapy for a central venous catheter-associated MRSA bacteraemia.

Conclusions: Laboratory strategies to reduce morbidity associated with hGISA should focus on testing for hGISA in bacteraemic (rather than colonizing) MRSA isolates in patients with recurrent S. aureus bacteraemia following glycopeptide exposure.

Keywords: MRSA, screening, teicoplanin, vancomycin

Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) infection is commonly treated with a glycopeptide. Unfortunately, glycopeptide therapy has been associated with the emergence of MRSA with reduced glycopeptide susceptibility.1 Reduced glycopeptide susceptibility takes three forms: glycopeptide-resistant S. aureus (GRSA; vancomycin/teicoplanin MIC > 8 mg/L); glycopeptide-intermediate S. aureus (GISA; vancomycin/teicoplanin MIC 8 mg/L) (BSAC criteria, 2009);2 and heterogeneous GISA (hGISA). The hGISA classification is given to isolates having a subpopulation with intermediate resistance. The clinical impact of hGISA is uncertain, but it may precede the GISA phenotype.3 If infection rates of S. aureus with reduced glycopeptide susceptibility increase it will be important to devise effective clinical responses. Given that screening for MRSA has been effective at controlling the spread of MRSA colonization and subsequent infections,4 it is important to consider if screening for S. aureus with reduced glycopeptide susceptibility may also be of benefit. Colonization with S. aureus with reduced glycopeptide susceptibility and subsequent infection has not, however, been shown. If this association were demonstrated, then detection and eradication of such colonization would need consideration. We tested colonizing and bacteraemic S. aureus isolates to identify those with and without reduced susceptibility to glycopeptides. These S. aureus isolates were characterized by molecular methods to determine if the colonizing...
and bacteraemic *S. aureus* with reduced glycopeptide susceptibility were related.

**Methods**

**General methods**

*S. aureus* isolates were from patients receiving care between 2004 and 2006 in three hospitals in Liverpool, UK. Isolates were from MRSA colonization screens and from blood cultures. The screen isolates were prospectively tested and blood culture isolates retrospectively tested for reduced glycopeptide susceptibility. An exception was three blood culture isolates with reduced susceptibility to glycopeptides identified prospectively by clinical laboratory investigations. *S. aureus* isolates were identified by slide coagulase testing, DNase production and biochemical testing, and stored at −70°C. Case records of patients with *S. aureus* bacteraemia demonstrating reduced glycopeptide susceptibility were examined to determine the patient’s clinical progress. This work was approved by the UK National Research Ethics Committee as service evaluation.

**Glycopeptide susceptibility testing**

**Agar incorporation method**

*S. aureus* isolates were screened for reduced glycopeptide susceptibility by an agar incorporation method; 5 μL of a 1.0 McFarland suspension was inoculated onto brain heart infusion agar (BHIA: BBL, Becton Dickinson) containing 8 mg/L teicoplanin or 6 mg/L vancomycin (incubation: 35°C/48 h/air). Isolates growing on these agars were tested by the MacroEtest method.

**MacroEtest method**

BHIA was inoculated with a 250 μL suspension (turbidity equivalent to that of a 2 McFarland standard) of *S. aureus* with vancomycin and teicoplanin Etest strips (bioMérieux, Marcy l’Etoile, France) placed on the agar (incubation: 35°C/48 h/air). Isolates with a MacroEtest reading of ≥8 mg/L to vancomycin and teicoplanin or ≥12 mg/L to teicoplanin were considered as having reduced glycopeptide susceptibility. The reduced susceptibility this method detects includes hGISA, GISA and GRSA. To define the type of susceptibility, Etest MICs were determined.

**Etest MIC method**

Etest MICs were determined according to the manufacturer’s instructions (bioMérieux). An isolate with reduced glycopeptide susceptibility by the MacroEtest method, but an Etest MIC of teicoplanin or vancomycin of ≥4 mg/L/8 mg/L was considered to be an hGISA/GISA, respectively (BSAC criteria, 2009).

**PAP–AUC method**

To confirm the hGISA classification, population analysis profile–AUC (PAP–AUC) analysis, a gold standard for identifying hGISA, was completed according to the methods of Wootton et al. PAP–AUC analysis generates a graphical representation of *S. aureus* growth in the presence of a range of vancomycin concentrations. The AUC of the test strain is compared with that of an hGISA control strain. PAP–AUC criteria define an hGISA as having a PAP–AUC ratio between 0.9 and 1.29. This method uses vancomycin, so does not define a heterogeneous teicoplanin-intermediate *S. aureus* (hTISA), which can occur independently of a heterogeneous vancomycin-intermediate *S. aureus* (hVISA). PAP–AUC was therefore also determined using teicoplanin.

**Molecular characterization**

PFGE was completed according to the HARMONY protocol, and staphylococcal protein A (spa) typing by the methods of Shutt et al. and Murchan et al. *Staphylococcal protein A* is a cell wall protein whose coding gene has a high rate of mutation, giving spa typing a high resolution, >5000 types identified (t001 to ~t5000).

**Results**

**MRSA colonizing isolates**

MRSA was isolated from 2550 patients, and 86 patients had an isolate with a MacroEtest reading of ≥12 mg/L to teicoplanin. Of these 86 patients, four had a blood culture with MRSA isolated within 2 weeks of the MRSA screen. None of these blood culture isolates had a MacroEtest reading of ≥12 mg/L to teicoplanin.

**Blood culture isolates**

In total, 5 of 201 MRSA blood culture isolates were determined to have a MacroEtest reading of ≥12 mg/L to teicoplanin. Three isolates were from patients who had previously been identified by the clinical laboratory to have reduced glycopeptide susceptibility. All five patients had an MRSA screen within 2 weeks of the blood culture isolate, four of which isolated MRSA. None of these four patients had MRSA isolated from the colonization screen with reduced glycopeptide susceptibility.

**Classification of glycopeptide susceptibility**

Standard MIC testing was completed on 15 isolates with a MacroEtest of ≥12 mg/L to teicoplanin (3 blood culture isolates and 12 MRSA screen isolates). Six isolates had an MIC of 4 mg/L to teicoplanin and ≤2 mg/L to vancomycin. Nine isolates had an MIC of ≤2 mg/L to teicoplanin and vancomycin. Therefore, none of these 15 isolates fulfilled the BSAC criteria for a GISA. Six of these isolates underwent PAP–AUC testing; one blood culture and five MRSA screen isolates. Two out of the six isolates (MRSA screen-derived isolates) were classified as hGISA or, more specifically, hVISA, using PAP–AUC analysis with vancomycin. PAP–AUC using teicoplanin showed that all six isolates tested had greater AUC values compared with Mu3 and 55173, hVISA and TISA reference strains, respectively (Figure 1). The combination of the standard MIC testing and PAP–AUC therefore confirmed that all six isolates were hTISA.

**Kanamycin characterization**

Kanamycin resistance was used to differentiate EMRSA-15 (kanamycin susceptible) from EMRSA-16 (kanamycin resistant), the two MRSA epidemic clones prevalent in Liverpool hospitals. PFGE confirmed that this method correctly identified EMRSA-15 and EMRSA-16 (data not shown). spa typing was also consistent with this EMRSA designation; EMRSA-15 spa type t032 and EMRSA-16 spa type t018. The majority of colonizing MRSA in Liverpool during the study period were EMRSA-15 (85%). In total, 82 of the 86 screen isolates identified as having reduced glycopeptide susceptibility were EMRSA-16 and 4 were EMRSA-15. Of five blood culture isolates with reduced glycopeptide susceptibility, three were EMRSA-15 and two were
EMRSA-16. The molecular characterization failed to identify differences between a selection of EMRSA-15 hGISA and non-hGISA isolates and EMRSA-16 hGISA and non-hGISA isolates (data not shown).

Clinical data
Of the five patients who had hGISA identified in a blood culture, four had previously received teicoplanin in response to MRSA in an earlier blood culture. All these four bacteraemias were central venous catheter infections. Two patients were receiving teicoplanin and two had previously received teicoplanin at the time hGISA was isolated. These four isolates were identified 12, 12, 30 and 180 days following the primary bacteraemia. The isolates identified 180 days apart were clinically and molecularly linked. The primary bacteraemia isolates from three of four patients who developed hGISA were available and did not demonstrate reduced glycopeptide susceptibility.

Discussion
In this study, 3.4% and 2.5% of patients colonized or bacteraemic with MRSA, respectively, had reduced susceptibility to glycopeptides. The reduced susceptibility was classified as hGISA according to BSAC criteria. However, criteria for classifying glycopeptide resistance vary. If European Committee on Antimicrobial Susceptibility Testing (EUCAST) classifications had been used, with resistance defined as vancomycin/teicoplanin MIC of >2 mg/L, many of our hGISA isolates would be classified as GISA. The rates of hGISA identified within our MRSA isolates are estimates only because the sensitivity of the teicoplanin agar incorporation method is not known. The vancomycin agar we used was shown by Wootton et al. to have a poor sensitivity but high specificity for the detection of hGISA/GISA, something we also found. The majority of our hGISA isolates were identified by the teicoplanin screening agar alone.

Molecular characterization of hGISA colonizing strains showed that 82 of the 86 isolates were EMRSA-16; surprising given that MRSA colonization in Liverpool is mainly with EMRSA-15. The characterization of hGISA isolated from blood cultures identified both EMRSA-15 (3/5) and EMRSA-16 (2/5). The presence of a colonizing hGISA in an MRSA screen did not predict hGISA bacteraemia. Also, hGISA colonization was not detected in patients with hGISA bacteraemia. Four of five hGISA bacteraemias were associated with prior glycopeptide therapy, suggesting that recurrent bacteraemia in the face of glycopeptide therapy is an important indicator of hGISA isolates. This study does not therefore support a policy of screening for colonization of S. aureus with hGISA, and subsequent eradication, as a means of preventing bacteraemia by these isolates.

Acknowledgements
We thank the staff of the Epidemiology Laboratory, Royal Liverpool and Broadgreen University Hospital Trust for their assistance in this study.

Funding
This work was supported by the University of Liverpool.

Transparency declarations
None to declare.

References