Case Report

In vivo development of heterogeneous glycopeptide-intermediate *Staphylococcus aureus* (hGISA), GISA and daptomycin resistance in a patient with meticillin-resistant *S. aureus* endocarditis

Andrew Kirby,^{1,2} Kavya Mohandas,¹ Caroline Broughton,² Timothy J. Neal,¹ Godfrey W. Smith,¹ Pearl Pai³ and Carlos Nistal de Paz¹

Correspondence
Andrew Kirby
amk@liv.ac.uk

¹Department of Medical Microbiology, Royal Liverpool & Broadgreen University Hospital Trust, Prescot Street, Liverpool, Merseyside L7 8XP, UK

²Department of Infection & Host Defence, Liverpool University, 8th Floor Duncan Building, Daulby Street, Liverpool L69 3GA, UK

³Department of Nephrology, Royal Liverpool & Broadgreen University Hospital Trust, Prescot Street, Liverpool, Merseyside L7 8XP, UK

We report a patient who developed a meticillin-resistant *Staphylococcus aureus* (MRSA) central venous catheter infection complicated by infective endocarditis. The patient was initially treated with glycopeptides, which led to the development of heterogeneous glycopeptide resistance, the detection of which required the use of a macro Etest screening test. Subsequently, the causative strain, confirmed by PFGE as a UK epidemic MRSA-15, was treated with daptomycin, and again resistance developed *in vivo*. The development *in vivo* of resistance to both these agents suggests that the resistance mechanisms may be associated. We suggest that the clinician managing MRSA infection should anticipate daptomycin resistance when reduced glycopeptide susceptibility is detected.

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Case report

A 41-year-old male with worsening renal function of unknown aetiology was admitted to the Royal Liverpool University Hospital. A Tesio catheter (double lumen and tunnelled) was inserted in the internal jugular vein for dialysis. A week later, pus was noted at the exit site of the Tesio from which meticillin-resistant *Staphylococcus aureus* (MRSA) was isolated and for which intravenous (i.v.) vancomycin was given. The infection appeared to resolve and the catheter remained *in situ*.

The following month, on 26 March 2005, he was readmitted with rigors, an inflamed exit site and temporary visual loss. MRSA was isolated from blood cultures taken through the catheter. This and subsequent isolates were speciated using standard methods. Antimicrobial susceptibilities were completed using the British Society for Antimicrobial Chemotherapy (BSAC) disc diffusion method (BSAC, 2007). This isolate was sensitive to

rifampicin, fusidic acid, trimethoprim, linezolid and gentamicin and resistant to clindamycin, ciprofloxacin and meticillin. Despite a diagnosis of endocarditis, a transthoracic echocardiogram (TTE) was not performed. The Tesio catheter was removed and the patient was started on i.v. teicoplanin (until 10 May) and fusidic acid (doses and duration unknown where not stated). No MRSA was isolated from 30 MRSA screens (nose, throat, axilla and groin) taken from this point onwards.

On 1 July 2005, MRSA was isolated from blood cultures. Treatment was commenced with i.v. vancomycin and oral rifampicin. Glycopeptide susceptibility results for this and subsequent isolates are shown in Table 1. Due to difficulty in establishing i.v. access, his Tesio catheter (inserted June 2005) was not removed. Instead, vancomycin lock therapy of the catheter lumen was started in addition to i.v. therapy. After 4 weeks, MRSA was again isolated from blood cultures. A TTE demonstrated no cardiac vegetations and a transoesophageal echocardiograph (TOE) was declined by the patient. i.v. teicoplanin was given for 6 weeks with trough antibiotic levels maintained at 40–60 mg l⁻¹. Oral fusidic acid was also given for the first 4 weeks and the catheter was removed. Peritoneal dialysis

Abbreviations: BSAC, British Society for Antimicrobial Chemotherapy; hGISA, heterogeneous glycopeptide-intermediate *Staphylococcus aureus*; i.v., intravenous; MRSA, meticillin-resistant *S. aureus*; TOE, transoesophageal echocardiograph; TTE, transthoracic echocardiogram.

Table 1. MICs (mg l⁻¹) of the MRSA isolates (completed in duplicate and retrospectively) from the patient

For comparison, BSAC (2007) MIC breakpoints (mg l⁻¹) for staphylococcal spp. are as follows. Teicoplanin: >8 resistant, 8 intermediate, ≤4 sensitive; vancomycin: >8 resistant, 8 intermediate, ≤4 sensitive; daptomycin: >1 resistant, ≤1 sensitive; linezolid: >4 resistant, ≤4 sensitive. Results in italics prospectively reported by the Antibiotic Resistance Monitoring & Reference Laboratory (ARMRL) for England and Wales, Colindale.

Isolate	Teicoplanin		Teicoplanin macro Etest		Vancomycin		Vancomycin macro Etest		Daptomycin		Linezolid	
March 05	1.0	0.5	3.0	3.0	1.0	1.0	3.0	3.0	0.25	0.025	0.75	0.075
July 05	0.5	0.5	3.0	3.0	1.0	1.5	3.0	2.0	0.5	0.25	1.5	2.0
December 05	1.5	2.0	6.0	6.0	1.0	1.0	6.0	3.0	0.5	0.75	1.0	1.5
May 06	8.0	8.0	12	16	3.0	3.0	6.0	4.0	3.0	3.0	0.75	2.0
May 06	6.0	6.0	16	12	3.0	3.0	6.0	4.0	6.0	3.0	1.5	1.5
December 06	16				4.0				4.0			

was attempted but failed, leading to arterio-venous fistula dialysis by October.

The patient was well until December 2005, when MRSA was again isolated in blood cultures. This isolate was screened for heterogeneous glycopeptide-intermediate *S. aureus* (hGISA) resistance, a form of glycopeptide intermediate resistance. Here a subpopulation of a strain demonstrates reduced susceptibility to glycopeptides. The screening was completed using a macro Etest method [2 McFarland inoculum, 48 h incubation on brain heart infusion agar (Becton Dickinson) at 35 °C] which identifies the resistant subpopulation. A teicoplanin MIC of 12 mg l⁻¹ by this method provides evidence of hGISA (Voss *et al.*, 2007). This was obtained at the time of testing though in our retrospective assessment a macro Etest MIC of only 6 mg l⁻¹ was found (Table 2). [Changes in MICs for hGISA isolates have been reported elsewhere (Boyle-Vavra *et al.*, 2000).] The isolate was confirmed as hGISA by population analysis profile–area underneath the curve analysis (PAP–AUC). PAP–AUC is the ‘gold standard’ for detecting hGISA. Here the AUC of the test *S. aureus* is divided by the corresponding AUC for Mu 3 (Wootten *et al.*, 2001), an hGISA type strain. A ratio of ≥0.9 is consistent with hGISA. Our isolate had a PAP–AUC ratio of 0.95. Following this, oral linezolid at 600 mg b.d. was commenced. The source of infection was not identified despite investigation including a TOE.

On 26 January 2006, day 45 of linezolid treatment, blood cultures grew MRSA sensitive to linezolid. Linezolid treatment was continued with the addition of gentamicin for 3 weeks (this isolate was fusidic acid-resistant; Table 2). A TOE performed in February 2006 showed two vegetations on the mitral valve. Antimicrobial therapy was changed to daptomycin 6 mg kg⁻¹ every 48 h, oral rifampicin 600 mg b.d. and 80 mg i.v. gentamicin post-dialysis for the first 2 weeks. In April, a repeat MRSA bacteraemia occurred. This isolate was still sensitive to daptomycin, leading to the addition of gentamicin.

On 9 May 2006, the patient developed rigors and dysphasia. Blood cultures were positive for MRSA which was resistant to daptomycin by Etest MIC at 3 mg l⁻¹ (all MIC values were derived using the Etest MIC method: 0.5 McFarland; Mueller–Hinton agar for 24 h at 35 °C). A computerized tomography scan of the head revealed an infarct, a presumed embolus from his infected endocardial vegetations. With the failure of daptomycin and linezolid, a regime based on trimethoprim 200 mg b.d. and rifampicin 600 mg b.d. was started. In addition, despite the MIC for glycopeptides of the latest MRSA strain, now confirmed as GISA with an MIC of 8 mg l⁻¹, with our experience of using high-dose teicoplanin, it was decided to administer teicoplanin with the aim of obtaining levels sufficient to negate the isolate’s increased MICs. Hence, i.v. teicoplanin at 1.6 g daily for 1 week, then approximately every 48 h,

Table 2. Antimicrobial susceptibilities of the patient’s MRSA isolates according to BSAC methods

S, Sensitive; R, resistant.

Isolate	Ciprofloxacin	Gentamicin	Erythromycin	Fusidic acid	Meticillin	Mupiricin	Rifampicin	Tetracycline	Trimethoprim
March 05	R	S	R	S	R	S	S	S	S
July 05	R	S	R	S	R	S	S	S	S
December 05	R	S	R	R	R	S	S	S	S
May 06	R	S	S	S	R	S	S	S	S
May 06	R	S	S	S	R	S	S	S	S
December 06	R	S	S	S	R	S	R	S	S

was prescribed, guided by levels. Throughout this period, teicoplanin pre-dose levels were always over 40 mg l⁻¹ and often >80 mg l⁻¹. i.v. gentamicin was also given for 2 weeks.

Four weeks into treatment, a TTE showed no vegetations and mitral valve replacement was performed. Post-operatively, treatment with i.v. teicoplanin 1.6 g every 48 h, oral rifampicin 600 mg b.d. and oral trimethoprim 200 mg b.d. was continued until 18 September 2006, a total of 8 weeks.

Isolates were available from March 2005 to May 2006, and PFGE was used to investigate their clonality (Harmony Method; Murchan *et al.*, 2003). This demonstrated that the isolates were one clonal type by PFGE (Fig. 1). Comparison with NCTC strain 8325 and the molecular mass marker (data not shown) identified the strain as a UK epidemic MRSA-15, the most prevalent strain of MRSA in our institution.

The patient remained asymptomatic for 6 months, when in December 2006 he presented again with rigors. Blood cultures were taken from which MRSA was isolated. A TOE showed no evidence of endocarditis but a radiolabelled

white cell scan suggested osteomyelitis of the humerus, not at the site of the fistula. i.v. tigecycline (MIC 0.094 mg l⁻¹) was given for a period of 2 months and clindamycin for 1 month, the isolate testing sensitive to clindamycin. The patient responded both clinically and radiologically and has remained infection-free since. Fig. 2 gives an overview of this case history.

Discussion

The MRSA infection treated with glycopeptides in this report developed, *in vivo*, hGISA prior to developing GISA in response to this glycopeptide therapy. hGISA populations are those where approximately one subclone in every 1 × 10⁶ is able to grow in the presence of a higher concentration of a glycopeptide. The concern is that treatment will fail to eradicate the resistant subpopulation, which will fill the niche left by the killed susceptible population, leading to the clinical failure of glycopeptide therapy. The outcomes in hGISA infections were recently reviewed by Falagas *et al.* (2008). The evidence was inconclusive with a limited number of retrospective studies showing both adverse and no adverse outcomes. There is therefore still a need for prospective evaluation of outcomes in *S. aureus*-infected glycopeptide-treated patients.

In view of the hGISA/GISA resistance, this patient was treated with daptomycin, which led to the development of daptomycin resistance *in vivo*. Sharma *et al.* (2008) also found that in patients with *S. aureus* infection, mainly endocarditis, treated with daptomycin, development of daptomycin resistance was not an uncommon finding.

The mechanisms of hGISA, GISA and daptomycin resistance are still being investigated and may vary between strains. A proposed mechanism involves the upregulation of peptidoglycan synthesis leading to hGISA. In the presence of this upregulation, a further mutation allows significant cell wall thickening, causing a GISA phenotype (Neoh *et al.*, 2008). The GISA phenotype, as we found, has been shown before to be associated with daptomycin resistance (Vikram *et al.*, 2005; Hayden *et al.*, 2005). It is suggested that daptomycin, the bactericidal effects of which are due to binding to the bacterial cell membrane, which causes an efflux of potassium ions from the cell and subsequent cell death (Silverman *et al.*, 2003), is prevented from exerting its anti-staphylococcal action by the thickened cell wall (Jones *et al.*, 2008). The thickened cell wall, with abundant daptomycin binding sites, sequesters and blocks daptomycin, preventing its action (Cui *et al.*, 2003). *In vitro* studies have shown an association of reduced susceptibility to daptomycin with reduced susceptibility to glycopeptides (Sakoulas *et al.*, 2006; Patel *et al.*, 2006).

Thus our case highlights that in patients with MRSA infection who demonstrate hGISA or GISA, which may require use of the macro Etest MIC for detection, clinicians

Fig. 1. PFGE profiles of the MRSA isolates. Dates of isolates: A, 03/2005; B, 07/2005; C, 07/2005; D, 12/2005; E, 05/2006; F, 05/2006; G, 05/2006.

Fig. 2. Schematic of significant clinical events, clinical management and *in vitro* susceptibility tests. CVC, central venous catheter; IE, infective endocarditis; vanc, vancomycin; rf, rifampicin; fd, fusidic acid; teic, teicoplanin; trim, trimethoprim; gent, gentamicin; MVR, mitral valve replacement.

should expect daptomycin resistance to develop on daptomycin therapy.

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