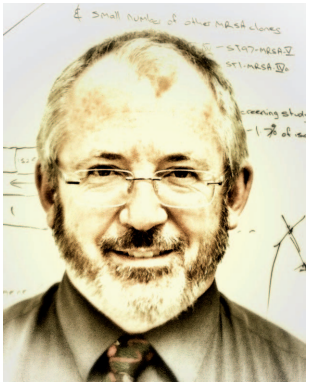
VISA and hVISA in hospitals



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***Staphylococcus aureus* is noted for its clinical spectrum of disease ranging from asymptomatic colonisation to overwhelming sepsis and death and for its ability to become resistant to antibiotics. Resistance to beta-lactams, methicillin resistance, was first described 50 years ago, becoming a clinical problem in hospitals in the 1970s and the community in the 1990s. MRSA strains that originated in hospitals are usually also resistant to most of the non-beta-lactams as well, leaving vancomycin as the main parenteral drug to treat serious MRSA infections, with the role of new drugs like daptomycin and linezolid not well defined. MRSA strains can exhibit low-level resistance to vancomycin (vancomycin-intermediate *S. aureus* [VISA]), probably due to a thickened cell wall, which results in the trapping of vancomycin away from the active site of the septum in dividing cells. Detecting this resistance is difficult as multiple genetic pathways lead to this resistance, obviating a molecular test, forcing reliance on phenotypic tests, all of which have issues with sensitivity, specificity and cost. Mortality of bloodstream infection correlates with vancomycin MIC so in this situation the MIC should be determined by Etest or microbroth dilution especially if endocarditis is present. Detection of resistant subpopulations (heterogeneous vancomycin-intermediate *S. aureus* [hVISA]) can be done with the expensive and time-consuming population analysis profile (PAP) but it is unclear if this confers additional therapeutic information.**

Types of vancomycin resistance in *Staphylococcus aureus*

There are two essential types of reduced vancomycin susceptibility in *S. aureus*: (1) that conferred by cell wall thickening resulting in a small rise in the MIC and/or the presence of subpopulations with a modestly elevated MIC[1]; and (2) that conferred by horizontal gene transfer of the *vanA* gene complex from vancomycin-resistant enterococci (VRE) to *S. aureus*, causing a more marked rise in the vancomycin MIC and clinical failure[2].

The Clinical and Laboratory Standards Institute (CLSI) reduced the breakpoints for vancomycin resistance in 2006 in response to the evidence that modest elevations of MIC were associated with a reduced likelihood of clinical response, and currently define vancomycin-susceptible *S. aureus* (VSSA) strains as having a MIC $\leq$4 mg/L, VISA as having MICs 4–8mg/L, and vancomycin-resistant *S. aureus* (VRSA) strains as having an MIC $\geq$16[1]. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) defines VSSA as strains with an MIC $\leq$2 and VRSA as having an MIC $\geq$4, with no intermediate division[1].

The first described clinical isolate of VISA was the so-called 'Mu50' strain, isolated from a child with an MRSA sternotomy infection that failed to respond to vancomycin but responded to debridement and arbekacin plus ampicillin/sulbactam; the microbroth vancomycin MIC was 8 mg/L[3].

Mu50 and subsequent VISA isolates exhibit common characteristics (Figure 1)[1]. They grow more slowly than VSSA, exhibit pleomorphic colonial morphology, have reduced or delayed coagulase, demonstrate thickened cell walls on electron microscopy, have alterations in cell wall metabolism, reduced susceptibility to lysostaphin and decreased autolysis.

hVISA strains usually have a vancomycin MIC in the CLSI and EUCAST susceptible ranges but possess subpopulations with a raised MIC, detectable by performing a PAP, where serial 10-fold dilutions of a suspension of *S. aureus* are plated on a series of agar plates containing increasing dilutions of vancomycin[1]. This was first described by Hiramatsu's group on the so-called 'Mu3' strain[4], and Wootton *et al*. refined this by using a series of plates with doubling concentrations of vancomycin and serial 10-fold dilutions of the inocula with Mu3 as the control, generating the categories of VSSA (PAP-AUC ratio test: Mu3 <0.9), hVISA (ratio 0.9–1.35) and VISA (ratio >1.35) (Figure 2)[5].

Just over a dozen patients to date have had MRSA isolated that possess the *vanA* gene complex[2]. Most are US origin; none have been reported from Australasia to date. The US patients are older with multiple co-morbidities, were colonised with *vanA* VRE and

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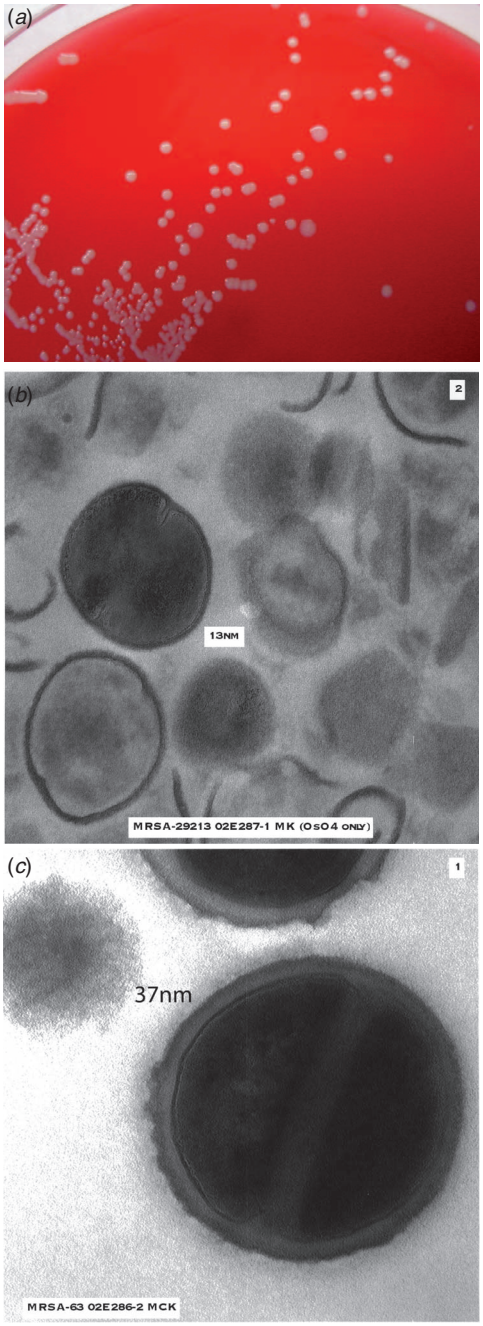

Figure 1. hVISA strains exhibit colonial pleomorphism, in this case on a horse blood agar plate (a). Electron microscopy of vancomycin-susceptible *Staphylococcus aureus* (b) and heterogeneous vancomycin-intermediate *Staphylococcus aureus* (c) strains show how the cell wall is thickened in the latter.

had high inoculum infections at sites difficult to penetrate with systemically administered vancomycin to which they have been extensively exposed. The *vanA* genes appear to have moved via plasmids from VRE into MRSA, conferring high-level resistance to vancomycin, therapeutic failure and major infection control issues.

Mechanism of resistance in VISA/hVISA

Hiramatsu has proposed the affinity-trapping hypothesis to explain vancomycin resistance in VISA/hVISA strains[6]. VISA/hVISA strains increase the cell wall thickness and vancomycin this binds to the outer layers of the cell wall and it is not available to penetrate into the deeper layers and thus interfere with cell division.

VISA/hVISA strains do not possess any elements of the *vanA*, *vanB, vanC*, etc., gene complexes found in enterococci that confer vancomycin resistance[1]. Many isolates have mutations in the *walKR* operon, which results in low activity, reduced autolysis and an increase in cell wall thickening[1]. The *mprF/fmtC* mutations result in reduced cell wall cross-linking[1]. There are also mutations in the *graR* genes and possible *vraR* as well, which results in upregulation of the cell wall stimulon[1].

Mwangi *et al*. applied whole genome sequencing to five sequential MRSA human blood culture isolates and found 35 point mutations across the five isolates, including genes involved in the cell envelope stress response, mutations in the *agr* quorum sensing system, the *walKR* wall operon and genes involved in peptidoglycan biosynthesis[7]. The group also detected *mprF yycG*, *rpoB* and *rpoC* gene mutations that were associated with daptomycin resistance (the patient was naïve to this drug). Cameron *et al*., performed comparative genomics in a seven sets of vancomycin exposed isogenic *S. aureus* pairs and found that serine/threonine phosphatase *stp1* contributes to reduce vancomycin susceptibility to vancomycin, in addition to previously described mutations such as *vraG*, *agaR*, *yvqF* and *rpoB*[8].

Clinical significance of VISA and hVISA

A recent systematic review and meta-analysis of the clinical significance of the vancomycin MIC in MRSA bloodstream infections compared mortality where the MIC was ≥1.5 µg/mL to isolates where the MIC was <1.5 µg/mL and found an odds ratio of death of 1.64 (95% CI 1.14–2.37) in the higher MIC group[9].

Holmes *et al*. compared patients with MRSA bloodstream infection where the vancomycin MIC was 2.0 or 3.0 versus MIC of ≤1.5 and found an all-cause 30-day mortality of 27% versus 12.5% ($P < 0.001$). The association with vancomycin MIC also occurred within the groups treated with flucloxacillin and in those with methicillin-susceptible *S. aureus*; the authors suggested that the raised vancomycin MIC might be an epiphenomenon[10].

Currently the clinical significance of hVISA status is unclear. van Hal and Paterson performed a systematic review and meta-analysis of the clinical significance of hVISA[11]. They found hVISA infections had a clinical failure rate 2.37 times that of those with VSSA (95% CI 1.53–3.67), but all-cause 30-day mortality in the two groups was not significantly different. Peleg *et al*. described the greater wax moth (*Galleria mellonella*) model in which *S. aureus* strains with

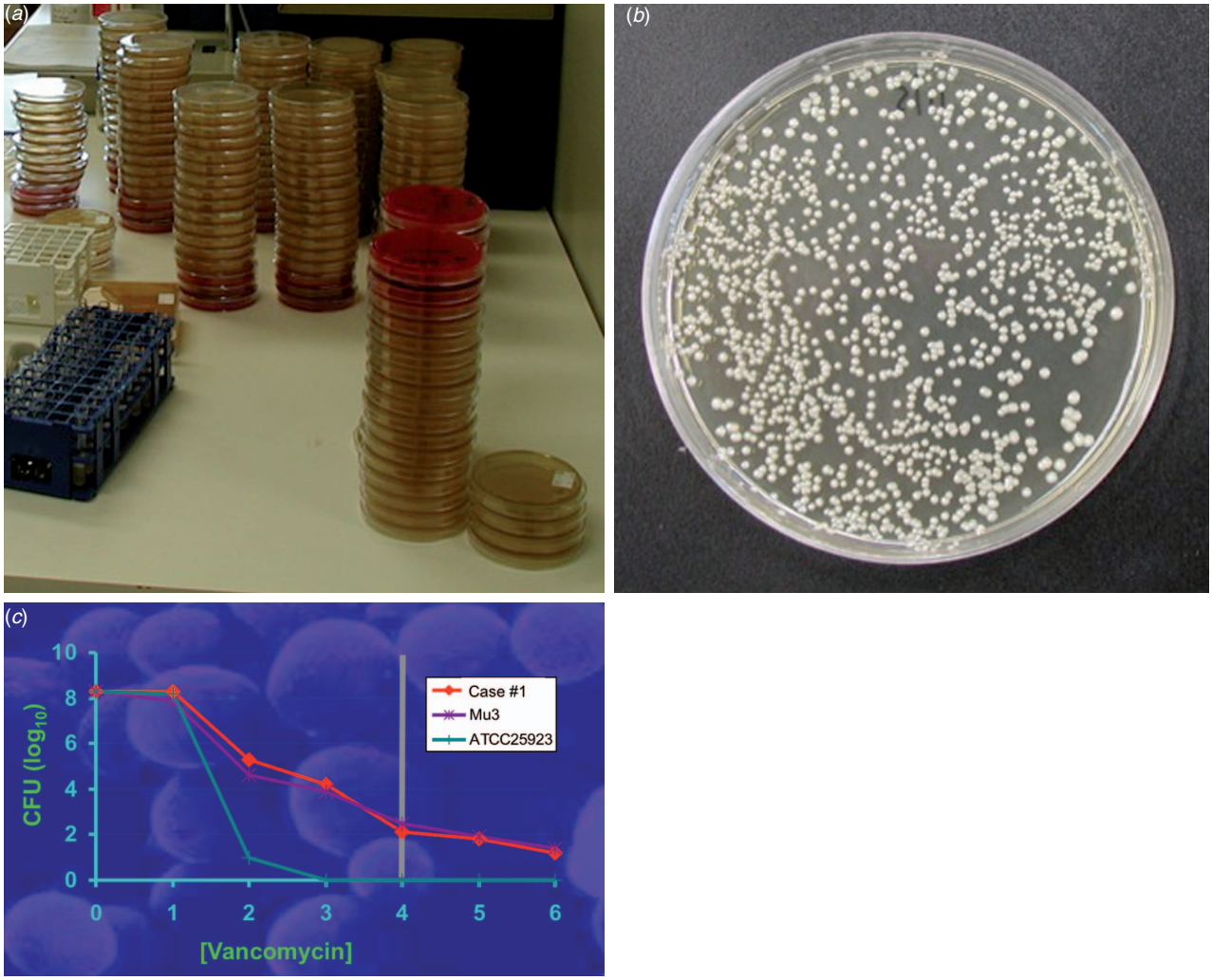

Figure 2. Population analysis profiling is currently the gold standard test for heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) in the absence of a genetic test or phenotypic test with high enough sensitivity and specificity. The method generally requires a large number of plates (a), generates several plates requiring colony counts (b), and then entry of the numbers into a spreadsheet to generate a PAP graph (c), calculate the area under the curves for the hVISA control (Mu3) and the test isolate. In this case the test isolate, our first hVISA strain, gave a curve almost indistinguishable from Mu3.

reduced susceptibility to vancomycin were associated with decreased pathogenicity and this correlated with decreased *agr* functional status[12].

Testing for VISA and hVISA

Testing can be done using a number of methods, as summarised in Tables 1 and 2.

Howden *et al*. in a comprehensive review outlined possible approaches to detection of VISA/hVISA[1]. If laboratory testing for VISA/hVISA is readily available, they suggest a laboratory-based approach, in which a vancomycin broth MIC could be used or alternatively modified Etest or an Etest GRD with confirmation of positives with a PAP, and if reduced susceptibility to vancomycin was demonstrated the treating doctor should consider daptomycin or linezolid. If VISA/hVISA testing is not readily available, a clinical approach could be adopted where a tardy clinical response might prompt the use of alternative agents such as daptomycin or linezolid. In clinical practice in the treatment of MRSA bacteraemia clinical failure often prompts the use of these agents before results of VISA/hVISA testing are available.

van Hal *et al*. compared different testing methodologies for detection of hVISA[14]. Four hundred and fifty-eight consecutive MRSA blood culture isolates were tested with PAP, macromethod Etest, glycopeptide resistance detection, GRD Etest and vancomycin MICs by Etest, by broth microdilution using CLSI criteria and Vitek2. Four hundred and fifty-eight isolates from 470 episodes of blood stream infection were analysed; 55 were hVISA and four were VISA by PAP, the latter 4 were excluded. The sensitivity and specificity of the various methods compared with PAP were Etest 91% and 66%, broth microdilution 89% and 84%, modified Etest 89 and 55%, broth microdilution (cutoff $\geq$2 mg/L) 82% and 97%, Etest (cutoff $\geq$2 mg/L) 71% and 94%, GRD Etest 71% and 94%, and the Vitek2

Table 1. Methods to detect VISA (based on van Hal and Fowler[13]).

Method	Pros	Cons	Comment
Broth microdilution MIC	Highly accurate	Labour intensive Usually batch	Reference method for MIC
Etest MIC	Easy, cheap	Reads 0–0.5 dilution higher than BMD	Most of the clinical papers correlating MIC to outcome used Etest MIC creep is usually associated with Etest
Etest glycopeptide resistance detection (GRD)	Easy, cheap More sensitive than Etest Can read at 24 h	Positives need to be confirmed	
Automated broth microdilution (Vitek, Phoenix, etc.)	Routinely used in many laboratories	Low inoculum and shorter incubation Insensitive Reads lower than BMD	
Screening agars	Easy, cheap	Variable sensitivity and specificity	
Disk diffusion		Insensitive and not recommended	Fails because large glycopeptide molecules diffuse slowly through agar
MALDI-TOF			One report so far; failed to work

Table 2. Methods to detect hVISA (based on van Hal and Fowler[13]).

Method	Pros	Cons	Comment
Population analysis profile (PAP)	Currently the reference method	Slow, labour intensive and costly	Presence of hVISA does not correlate with higher mortality in SAB
Screening agars	Easy and cheap	Insensitive and not specific	Decreasing concentration of glycopeptide in agar increases sensitivity at expense of specificity
Modified Etest (MET)	Easy and cheap	Sensitive but poor specificity	Increased inoculum and prolonged incubation selects out resistant subpopulations Result is not the MIC (Figure 3)

(MIC estimate ≥2 mg/L) 25% and 96%, respectively. PAP, modified Etest, GRDE test, broth microdilution, standard Etest and Vitek2 cost $70–$320, $29, $12, $8–$38, $15 and $18 per isolate, respectively. Etest tended to read higher and Vitek2 lower than BMD. Performing PAP on all the isolates would have 100% sensitivity and specificity (assuming PAP is the gold standard) but cost $32,000 to test all MRSA isolates in the study. Screening with Etest with cutoff MIC ≥1.5 confirming positives with PAP gave 98.9% sensitivity and cost ~$20,000. MET followed by PAP gave a sensitivity of 89% but cost ~$30,000. Other test combinations were cheaper but insensitive.

Holmes *et al*. recently summarised key points about the clinical and laboratory implications of the different types of vancomycin-reduced susceptibility[15]. For methicillin-susceptible *S. aureus* infections, β-lactams give better results than glycopeptides. For invasive MRSA infections, optimal vancomycin dosing and source control are pivotal. In this situation an MIC should be determined, preferably by

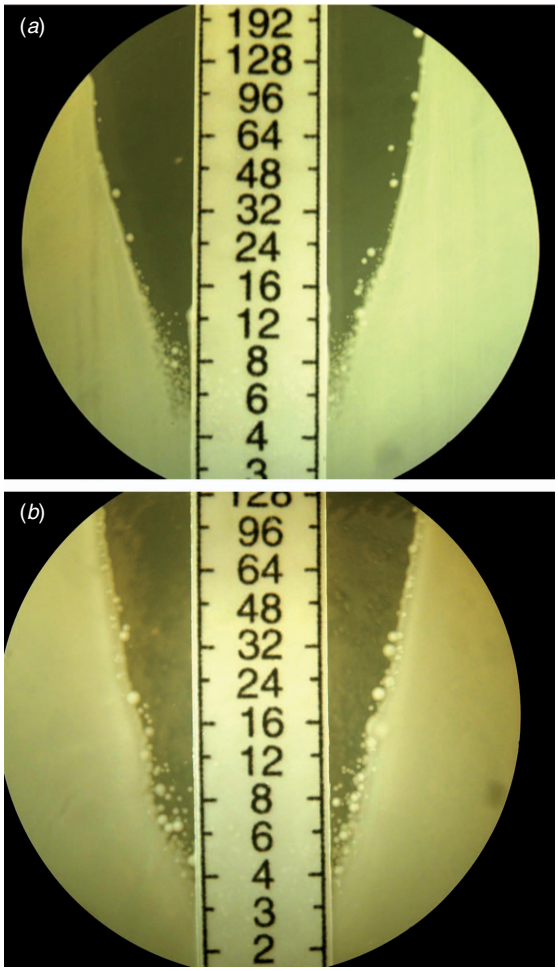

Figure 3. The macromethod Etest (MET) heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) screen uses a 2.0 McFarland inoculum and 48 hours of incubation to select resistant subpopulations. A positive is defined as a reading for teicoplanin ≥12, or if the teicoplanin MIC is ≥8 and the vancomyin reading is also ≥8. Note that these numbers are not the MIC. In this case, the teicoplanin (a) and vancomycin (b) readings are both ≥8; this is the strain whose PAP is shown in Figure 2c, where it was confirmed as a hVISA. Note also the appearance of small colonies close to the Etest strip, and the morphological variation in the colonies, which is also typical of hVISA strains.

BMD but Etest was satisfactory. If there was clinical failure of vancomycin and/or one of the recommended methods suggested reduced vancomycin susceptibility, daptomycin or linezolid should be considered. If the MIC ≥16 mg/L polymerase chain reaction testing for *vanA* and *vanB* genes should be performed as the isolate may be a VRSA.

MIC creep

van Hal and Fowler summarised the studies looking at MIC creep, which refers to the slow increase in the MIC of the MRSA isolates over period of time[13]. Creep is mostly demonstrated if Etest is used and not found in most studies using broth microdilution. Studies that demonstrated MIC creep tended to be single-centre studies. Typing was only performed in a small number of studies and where this was done, creep was demonstrated where there was a clonal emergence of strains with increased MICs, which probably explains the phenomenon.

Are VISA/hVISA strains less susceptible to daptomycin?

Daptomycin is a large molecular weight compound with a site of action in the cell wall, like vancomycin, and it has been hypothesised that VISA/hVISA strains might be less susceptible to daptomycin. A patient treated unsuccessfully with vancomycin but never treated with daptomycin, has a series of MRSA isolates from blood demonstrating increasing resistance to vancomycin and also daptomycin[16]. Sakoulas *et al*. found MRSA strains that were heterogeneously resistant to vancomycin were also heterogeneously resistant to daptomycin[17]. Another group found MRSA MICs to daptomycin and vancomycin were strongly correlated ($P > 0001$, χ^2 test)[18].

Conclusions

If there is clinical failure of glycopeptide treatment of MRSA infection, especially bacteraemia, generally daptomycin or linezolid will be selected before screening or definitive testing for VISA/hVISA can be performed. In the case of MRSA bacteraemia, especially if endocarditis or other deep focus is present, the MIC should be determined, either by Etest (not modified Etest which is a screen for hVISA) or broth microdilution.

Fortunately most MRSA infections do not involve bacteraemia. Vancomycin is cheap, generally well tolerated, has defined therapeutic drug monitoring strategies, and works well for the parenteral treatment of most MRSA infections.

Future directions

The clinical significance of hVISA needs to be defined. A cheap reliable test that predicts clinical failure with vancomycin is needed. The role of daptomycin, linezolid and the new anti-MRSA cephalosporin ceftaroline in serious MRSA infections needs to be clarified.

Acknowledgements

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Biography

Professor Iain Gosbell is the Foundation Professor of Infectious Diseases and Microbiology at the School of Medicine, University of Western Sydney, Australia. He described the emergence of community MRSA in south western Sydney, and subsequently developed research interests in the epidemiology of MRSA in the community and also in hospitals, and the detection of vancomycin resistance in MRSA. He established the Infection Prevention Unit at Liverpool Hospital in conjunction with their Infection Control Practitioners. Since commencing at UWS, he helped establish the Antibiotic Resistance and Mobile Elements Group which is now based at the Ingham Institute for Applied Medical Research at Liverpool. ARMEG consists of Professor Gosbell, Dr Slade Jensen, Dr Björn Espedido and Associate Professor Sebastiaan van Hal. Its mission is to research the genetics responsible for antibiotic resistance, especially vancomycin and daptomycin resistance in MRSA, plasmid biology of MRSA, and the contribution of bacterial biofilms to healthcare-associated infections. Professor Gosbell also promotes the importance of Microbiology and Infectious Diseases and the use of Information Technology in Medical Education. He was awarded the 2013 ASM bioMérieux Identifying Resistance Award.

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