commonly known as *spa* typing [8]. A recent technical development is high resolution melt (HRM) analysis. This is now available on many real-time PCR devices. Very recently, HRM was shown to be very effective for discriminating between *spa* alleles [9]. This method is ideal for combining with, for example, real-time PCR interrogation of the clonal complex defining SNPs.

In conclusion, the authors of this article have been involved in the development of typing methods using two different platforms – oligonucleotide array and real-time PCR/HRM analysis. However, there are significant commonalities between the two approaches as regards the use of multiple loci, and the emphasis on achieving consistency with other typing methods and the actual *S. aureus* population structure. The advantage of array-based methods is that they can interrogate a large number of targets. The advantage of real-time PCR based methods is that they are single step-closed tube, and can interrogate different classes of polymorphisms such as SNPs, binary markers and variable number tandem repeats. It is easy to envisage a convergence of these approaches with, for example, an on-chip amplification format that includes HRM analysis of the products.

Acknowledgements

PG acknowledges funding from the CRC for Diagnostics and Queensland Health Pathology Service, and also acknowledges Flavia Huygens for the preparation of Fig 2. FOB acknowledges funding from the National Health and Medical Research Council of Australia (NHMRC), the Department of Health of Western Australia and the Curtin University of Technology, WA.

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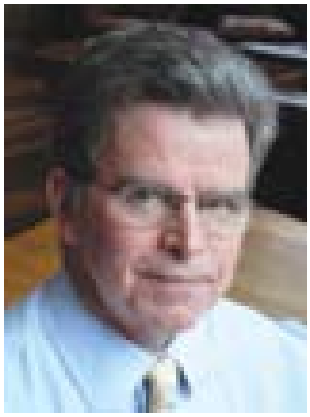
Detecting hVISA



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Rates of reduced vancomycin susceptibility (RVS) among isolates of methicillin-susceptible *Staphylococcus aureus* (MSSA), especially methicillin-resistant *S. aureus* (MRSA), have increased. Although vancomycin remains the therapeutic mainstay for MRSA, clinical response to vancomycin has been compromised by RVS among our hospital clones. Laboratories need simple procedures to detect these organisms. Whilst routine disc or MIC susceptibility testing is not reliable, some useful screening procedures are available.

The first vancomycin-intermediate *S. aureus* (VISA) isolate of MRSA was recognised in 1996 [1]. The first Australian isolate with RVS was reported as hetero-VISA (hVISA) in 2001 [2]. Isolates of hVISA contain subpopulations (10^{-5} to 10^{-6}) that grow at increased vancomycin concentrations. Patients with hVISA infections suffer persistent infection despite apparently adequate vancomycin therapy [3, 4]. Mortality is reported to be higher (63%) in patients with RVS strains versus 12% mortality in patients with susceptible strains [5].

Reduced susceptibility is also evidenced *in vitro*. Vancomycin MICs are increasing with higher proportions of MRSA, with MICs >1mg/L increasing from 26-70% over 4 years [6]; similar data exist

in the USA [7]. Likewise, the bactericidal activity of vancomycin on isolates from patients with persistent infection is often 100-fold less than isolates from patients with infections resolving with vancomycin [8].

The incidence of hVISA is significant. Examples of reported rates in French hospitals include 11% among 2300 isolates of MRSA [9], and 3.6% across 63 hospitals [10]. Perhaps not surprisingly, outbreaks of *S. aureus* with RVS have also been reported [9-12].

Laboratory detection of hVISA

Many studies have reported the degree of resistance to vancomycin to increase during glycopeptide therapy. Hence, the cultures of *S. aureus* or MRSA from patients receiving vancomycin for persistent infection, especially bacteraemia, should be further investigated. Cultures on blood agar regularly show variable colony morphology (size and pigmentation) among a small to a major proportion of colonies. This may take 48 hours incubation to reveal the slower growing resistant sub-population (Table 1). Microbroth MIC performed according to Clinical and Laboratory Standards Institute (CLSI) standards are useful for defining VISA (vancomycin MIC 4-8mg/L) and are of some use for hVISA.

Population analysis profile (PAP), used as the gold standard for identifying hVISA, measures proportions of a population that survive and grow at a range of vancomycin concentrations. The area under the growth curve is compared with that of a type strain of hVISA (Mu3) [13]. Unfortunately the test is labour intensive and takes a minimum of 3-5 days; hence it is not a routine diagnostic test.

The reliability of screening methods varies considerably and most have been used to detect hetero-glycopeptide Intermediate *S. aureus* (hGISA) rather than specifically hVISA or hetero-teicoplanin intermediate *S. aureus* (hTISA).

A recent multicentre trial (12 laboratories from the USA, Europe and Australia) compared two screening media and Macromethod Etest (MET) against PAP against 15 VISA/hVISA strains. The medium recommended by the Centre for Disease Control (CDC) for vancomycin screening – BHIA containing 6mg vancomycin/L (BHV6) spot inoculated with 10μL of a 0.5 McFarland suspension – had a sensitivity of 11% for detecting hGISA. In contrast, the European Antimicrobial Resistance Surveillance System (EARSS) recommended Mueller-Hinton agar – 5mg teicoplanin/L (MHT5) inoculated with 10μL of 2 McFarland suspension – had 80% sensitivity for hGISA and 76% specificity; MET had 69% sensitivity and 89% specificity [14].

Fitzgibbon [15] recently evaluated six different screening methods for hGISA using vancomycin PAP (vPAP) and teicoplanin PAP (tPAP) as gold standards [13]. The best performing medium was

Table 1. Definition of hVISA, VISA and VRSA.

	VSSA	hVISA	VISA (CLSI 2006)	VRSA (CLSI 2008)
Microbroth MIC (mg/L)	≤2	<4	4-8	≥16
Etest macromethod screen		≥8 vancomycin & teicoplanin or ≥12 teicoplanin		
Etest modified macromethod screen		>8 teicoplanin		
PAP-AUC [13]	<0.9 (usually <0.7)	≥0.9	≥1.30	

Table 2. Comparison of performance of screening media for hGISA/GISA.

Study	Screen medium*	% sensitivity for GISA/hGISA	% specificity for GISA/hGISA	Gold standard No. isolates tested
Wootton M *et al.* [14]	$BHV6_{0.5}$ $MHT5_{2.0}$ Macro Etest (MET)	11 80 69	97 76 89	vPAP triplicates of 15 strains, 3 controls
Fitzgibbon MM *et al.* [15]	$BHIT5_{0.5}$ $BHIT5_{2.0}$ $BHIT5_{0.5}$ plus mMET	100 100 99	57 27 84	vPAP and tPAP 3,189 isolates
Voss A *et al.* [16]	$MHV5_{0.5}$ $MHT5_{2.0}$ MET	25 90 99	59 92 93	vPAP quadruplicates of 25 strains

*Subscript indicates density of McFarland suspension used to inoculate media.

BHIA containing 5mg teicoplanin/L (BHIT5) when confirmed by modified macromethod Etest criteria of ≥8mg/L for teicoplanin (mMET). The BHIT5 was spot inoculated with 10μL of 0.5 McFarland suspension of a stationary phase culture and incubated for 24 and 48 hours. Growth of >1 colony was further investigated with mMET. Although this combined method (screen plus Etest) is very promising (sensitivity 99% and specificity 84%), they recommend confirmation by PAP. MHT5 combined with mMET had sensitivity and specificity of 96% and 73% respectively.

Another multicentre evaluation [16] of inter-laboratory reproducibility of GISA/hGISA screening reported MHT5 to have sensitivity and specificity for hGISA of 90% and 92%, 44% and 68% for BHIV6, 25% and 59% for MHV5 and 99% and 93% for MET (Table 2).

Others assessing bactericidal activity report a significant relationship between persistent bacteraemia and reduced killing of vancomycin (<2.5 Log_{10} killing over 24 hours) when compared with patients without persistent bacteraemia (p=0.025) [17]. Microbroth MIC (CLSI) also differentiated isolates with reduced susceptibility, patients with persistent bacteraemia having vancomycin MICs of 2mg/L compared to those with resolving bacteraemias with isolates with MICs of ≤1.0mg/L (p=0.019) [17].

Whilst many of the above screening methods are useful in detecting hVISA and VISA, simpler approaches that can be incorporated into routine susceptibility testing are still required to provide indication of reduced susceptibility in a clinically valuable timeframe. In the interim, $BHIT5_{0.5}$ followed by mMET and PAP is recommended.

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Tony Della-Porta retires from the *Microbiology Australia* Editorial Board

At the *Microbiology Australia* Editorial Board meeting at the Melbourne ASM conference, Dr Tony Della-Porta retired from the Board after 13 years of active service. Tony was one of the original members of the Editorial Board formed in 1995 under the chairmanship of Dr Dick Groot-Obbink to provide ASM members with a new-look journal, *Microbiology Australia,* edited by Joan Eyles.

Tony has been a very active contributor to the journal, guest editing six issues; on wildlife diseases (with John Mackenzie, May 1996), aquaculture (May 1997), the importation of agricultural diseases (July 2001), bioterrorism –Australia's response (May 2003), the advances in diagnostic techniques/ point of care testing (May 2006) and biomanagement and risk assessment (May 2008).

The ASM have certainly benefited from Tony's enthusiasm and dedication to *MA,* and he will be missed.