The British Society for Antimicrobial Chemotherapy

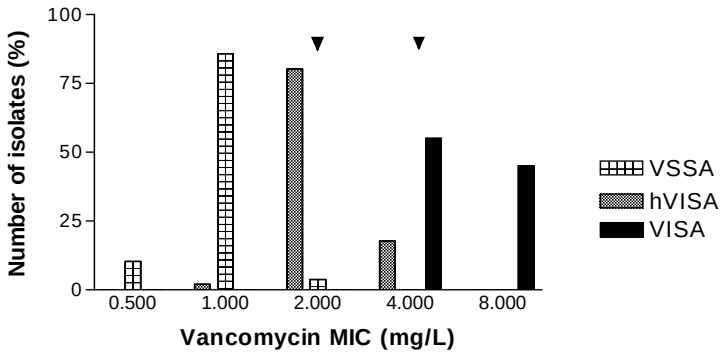
Susceptibility testing of *Staphylococcus aureus* to vancomycin

Susceptibility testing of *S. aureus* to vancomycin (and teicoplanin) is problematic.

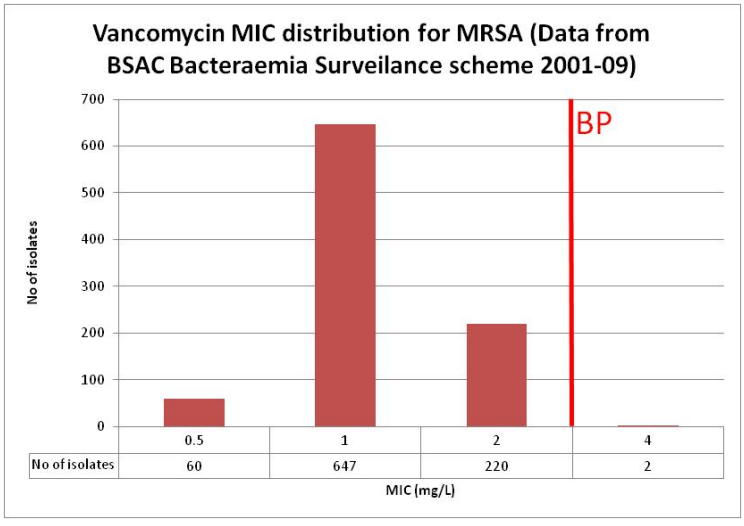
The main issues are:

- Uncertainty regarding the target for testing:
  High-level resistance to vancomycin with MICs of ≥32 mg/L (conferred by acquisition of *vanA* genes) is extremely rare. The majority of strains with reduced susceptibility have low-level resistance with MICs of 3-8 mg/L.
  The gold standard for predicting clinical failure is an MIC breakpoint. The current breakpoint for Vancomycin for *S. aureus* is S ≤2mg/L, R>2mg/L.
  Although there is some suggestion that isolates with MICs between 1-2 mg/L may respond less well to therapy, this is currently insufficient to warrant a reduction in the breakpoint.
  Isolates have been described that display heterogeneous low-level resistance to vancomycin (ie while the MIC is ≤2mg/L and gives a "sensitive" result, a sub-population of cells are present that can grow in the presence of higher vancomycin concentrations). Infections caused by these hVISA or hetero-VISA appear to respond less well to therapy with vancomycin, although it remains unestablished whether this is due to their hetero-resistance alone, or the fact that they tend to have slightly higher MICs.(5)

- MIC testing does not provide clear differentiation between Vancomycin –sensitive isolates (VSSA), hVISA, and VISA.
  Vancomycin MIC distributions for VSSA, hVISA, and VISA are shown in the graph below (from Wootton et al (2005)).(5)



- The wild-type population distribution of MICs for MRSA is extremely close to the clinical breakpoint of 2 mg/L.



- Minor modifications in testing methods can cause changes in the MIC measurement which may then cause a change in S/I/R categorisation. For example, slightly higher MICs may be observed when
    - testing is performed on Mueller-Hinton agar rather than IsoSensitest agar
    - a higher bacterial inoculum is used
    - a gradient strip is used

Testing

- Disc testing does not detect low-level resistance (and is unreliable at detecting high- level resistance).

- The reference standard for testing is an MIC performed using the ISO (International Standards Organisation) method using Mueller-Hinton Broth.
  The reproducibility of MIC testing is one doubling dilution either side of the MIC. Therefore it should be recognised that a proportion of isolates with MIC of 2 mg/L (sensitive) will sometimes test as 4 mg/L (resistant). This is inevitable variability inherent within the test.

- Gradient strips, when developed, should be calibrated against the ISO broth microdilution method and should therefore give equivalent results. However, slightly higher MICs have been observed with some Gradient Strips. It is therefore imperative that they are controlled with the specified control strains.

- Automated systems struggle to differentiate isolates with MICs around the susceptibility breakpoint. Some systems systematically overcall resistance, while others overcall sensitivity. (1)

- Screening methods for reduced vancomycin susceptibility.
  Various screening agars have been developed for the preliminary identification of reduced susceptibility. Of these, the method using Mueller-Hinton agar incorporating 5mg/L Teicoplanin has the best performance and inter-laboratory reproducibility. (4)
  Methods developed using either standard vancomycin and teicoplanin Etests with a high inoculum, (2) or a GRD (Glycopeptide Resistance Detection) Etest with a standard inoculums (6) have been shown to have reasonable sensitivity and specificity. Confirmation of hetero-resistance is definitively established with a Population Analysis Profile. (3) Isolates can be referred to Cardiff or North Bristol[1] if confirmatory testing is felt to be clinically indicated.

Recommendations

- Disc testing should NOT be used.

- An MIC-based method should be used.
  Whichever method is used, a control strain (ATCC 25923 or ATCC 29213) should be tested in parallel with each run of test organisms. The acceptable control MIC ranges (mg/L) are given in the table below.

	ATCC 25923	ATCC29213
Vancomycin	0.25 - 1	0.5 - 2

[1] Public Health Wales, Microbiology Cardiff, University Hospital of Wales, Heath Park, Cardiff, CF14 4XW.
Department of Microbiology, Lime Walk Building, Southmead Hospital Westbury–on-Trym, Bristol, BS10 5NB.

- If the control readings are outside the acceptable range, the results for the test organism should be treated with caution. If control readings are consistently outside the acceptable range, this suggests a systematic error which should be investigated. It should be noted that gradient strips have been observed to give higher readings (by half to one 2-fold dilution) than reference microbroth dilution methods.

- Laboratories may wish to target their testing of *S. aureus* to invasive or serious infections, or cases of treatment failure. This is supported by the rarity of resistant isolates in the UK (0.2%in BSAC Bacteraemia surveillance)

- Any isolates with an MIC of >8mg/L should be referred to a reference laboratory for confirmation.

Reference List

1. **Swenson, J. M., K. F. Anderson, D. R. Lonsway, A. Thompson, S. K. McAllister, B. M. Limbago, R. B. Carey, F. C. Tenover, and J. B. Patel**. 2009. Accuracy of commercial and reference susceptibility testing methods for detecting vancomycin-intermediate Staphylococcus aureus. J.Clin.Microbiol. **47**:2013-2017. doi:JCM.00221-09 [pii];10.1128/JCM.00221-09 [doi].
2. **Walsh, T. R., A. Bolmstrom, A. Qwarnstrom, P. Ho, M. Wootton, R. A. Howe, A. P. MacGowan, and D. Diekema**. 2001. Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J.Clin.Microbiol. **39**:2439-2444. doi:10.1128/JCM.39.7.2439-2444.2001 [doi].
3. **Wootton, M., R. A. Howe, R. Hillman, T. R. Walsh, P. M. Bennett, and A. P. MacGowan**. 2001. A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in Staphylococcus aureus in a UK hospital. J.Antimicrob.Chemother. **47**:399-403.
4. **Wootton, M., A. P. MacGowan, T. R. Walsh, and R. A. Howe**. 2007. A multicenter study evaluating the current strategies for isolating Staphylococcus aureus strains with reduced susceptibility to glycopeptides. J.Clin.Microbiol. **45**:329-332. doi:JCM.01508-06 [pii];10.1128/JCM.01508-06 [doi].
5. **Wootton, M., T. R. Walsh, and A. P. MacGowan**. 2005. Evidence for reduction in breakpoints used to determine vancomycin susceptibility in Staphylococcus aureus. Antimicrob.Agents Chemother. **49**:3982-3983. doi:49/9/3982 [pii];10.1128/AAC.49.9.3982-3983.2005 [doi].
6. **Yusof, A., A. Engelhardt, A. Karlsson, L. Bylund, P. Vidh, K. Mills, M. Wootton, and T. R. Walsh**. 2008. Evaluation of a new Etest vancomycin-teicoplanin strip for detection of glycopeptide-intermediate Staphylococcus aureus (GISA), in particular, heterogeneous GISA. J.Clin.Microbiol. **46**:3042-3047. doi:JCM.00265-08 [pii];10.1128/JCM.00265-08 [doi].