

Comparison of gradient strips for use in detection of *Staphylococcus aureus* isolates with reduced susceptibility to glycopeptides.

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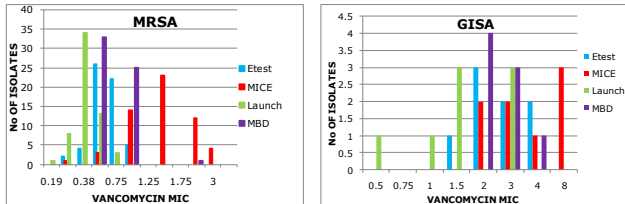
J. Richards, C. Estrada, L. Davies, M. Wootton, R A. Howe



Introduction

Reduced glycopeptide resistance in *Staphylococcus aureus* is an important clinical problem. Both homogenous and heterogeneous intermediate resistance to glycopeptides in *S. aureus* (GISA/hGISA) is difficult to detect using most standard disc susceptibility testing. Current advice (BSAC) recommends using MIC determination, which aids detection of GISA and hGISA. This can be performed using many techniques, including gradient strips. The reference method for MIC determination is Microbroth dilution (MBD) which is cumbersome for a busy diagnostic laboratory. Alternatively, gradient strip testing is quick and easy to perform. This study aims to compare the different commercial gradient strips of vancomycin and teicoplanin against a known set of hGISA/GISA and glycopeptide susceptible *S. aureus* (GSSA).

Fig 1. Frequency of MRSA and GISA MICs.



Methods

8 GISA, 48 hGISA and 59 GSSA were used. MIC determination was performed using Ettest (BioMerieux), MICE (Oxoid) and MIC test (Liofilchem/Launch diagnostics) strips plus MBD. All tests were performed on Mueller Hinton agar (MHA) as advised by the manufacturer. All gradient strips were compared on both MHA and Isosensitest agar (ISA) in control strains on 15 occasions; Mu3 (hGISA), Mu50 (GISA), ATCC25923 (GSSA) and ATCC29213 (GSSA). Geometric mean MICs (GeoMeanMIC) were calculated, and sensitivity (sn), specificity (sp) for the detection of GISA were compared for both vancomycin (VAN) and teicoplanin (TEIC).

Table 1: Results for GSSA/hGISA/GISA against VAN and TEIC

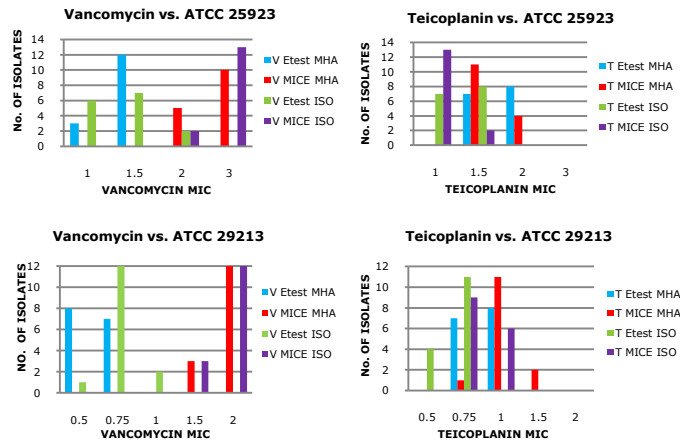
	VAN				TEIC			
	GeoMean GSSA/hGISA/GISA	sn	sp	GeoMean GSSA/hGISA/GISA	sn	sp		
Ettest	0.6 / 1.3 / 2.5	50	100	0.4 / 2.1 / 4.3	63	100		
MICE	1.4 / 2.4 / 4.1	87.5	93.3	0.3 / 1.8 / 3.9	63	100		
MIC test	0.4 / 0.9 / 1.6	37.5	100	0.7 / 1.8 / 4.2	63	100		
MBD	0.7 / 1.9 / 3.1	50	100	0.4 / 2 / 4	63	100		

Table 2 : Comparison of media using control strains.

VAN	ATCC 25923	ATCC 29213	Mu3	Mu50	sn	sp
	GeoMean MHA/ISA	GeoMean MHA/ISA	GeoMean MHA/ISA	GeoMean MHA/ISA	MHA/ISA	MHA/ISA
Ettest	1.4 / 1.3	0.6 / 0.8	1.5 / 1.5	4.0 / 2.7	50 / 36.7	100 / 100
MICE	2.6 / 2.8	1.9 / 1.9	1.5 / 1.5	4.0 / 3.9	46.7 / 50	66.7 / 56.7
MIC test	1.1 / 1.2	0.8 / 0.8	0.9 / 0.9	3.1 / 2.3	46.7 / 20	100 / 100

TEIC	ATCC 25923	ATCC 29213	Mu3	Mu50	sn	sp
	GeoMean MHA/ISA	GeoMean MHA/ISA	GeoMean MHA/ISA	GeoMean MHA/ISA	MHA/ISA	MHA/ISA
Ettest	1.8 / 1.3	0.9 / 0.7	7.7 / 6.0	8.0 / 8.0	100 / 100	100 / 100
MICE	1.6 / 1.1	1.0 / 0.8	3.8 / 3.3	5.1 / 5.1	100 / 96.7	100 / 100
MIC test	0.5 / 0.6	0.3 / 0.4	5.5 / 4.7	2.8 / 3.2	90 / 100	100 / 100

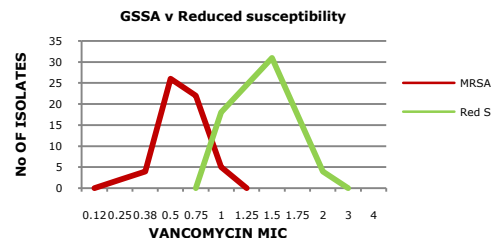
Figure 2: Frequency of 2 *S. aureus* control isolates on MHA and ISA.



Results

The results are summarised in Table 1. VAN GeoMeanMICs were 1.3/2.4/0.9 and 2.5/4.1/1.6 respectively for hGISA and GISA respectively compared with 1.9 and 3.1 for MBD. Sn & sp for detection of GISA were 50% & 100% for both Ettest and MBD, while sn was higher for MICE at 87.5% with reduced sp at 93.3%. The frequencies of VAN MICs in GSSA, hGISA and GISA (Figure 1) show comparable results for Ettest and MBD with higher MICs for MICE & lower MICs for MIC test strips. Comparison of replicate testing of two control GSSA strains (Table 2) ATCC 25923 & ATCC 29213 showed similar results for ISA and MHA. However for VAN, MICE gave noticeably higher readings than Ettest and MIC test lower (Figure 2). To highlight the difficulty in detecting hGISA, figure 3 shows the crossover of MICs for GSSA and isolates with reduced susceptibility.

Figure 3: MICs using Ettest of GSSA and isolates with reduced susceptibility.



Conclusions

No method, including the reference MBD method gave good sensitivity for detection of GISA. Of the gradient tests examined, Ettest gave equivalent results to MBD, while MICE had increased sensitivity, but reduced specificity and MIC test lower sensitivity. Results for control strains were not affected by media. Further work is required to establish an appropriate method for establishing reduced glycopeptides susceptibility in *S. aureus*.