



Introduction

Multidrug resistance is common among coagulase negative staphylococci (CoNS) and possesses a problem when treating CoNS infections. Vancomycin is often used to treat seriously ill patients with CoNS infections. Treatment failures with vancomycin have been shown to be due to the presence of staphylococci with intermediate and heterogeneous resistance to glycopeptides. This resistance cannot be detected with standard minimal inhibitory concentration (MIC) determination and therefore other methods have to be used. The gold standard method, population analysis profile – area under the curve (PAP-AUC), is too laborious to be used in a clinical microbiology laboratory. We therefore evaluated the macrodilution method with Etest (MME) and the glycopeptide resistance detection Etest (GRD) on clinical isolates of CoNS. These methods have shown good results and low intra- and inter laboratory variability when they have been evaluated on *S. aureus* [1, 2].

Materials and Methods

158 CoNS isolates from hospitalized patients and 25 CoNS isolated from patients undergoing revision surgery for prosthetic infection were included. Standard MIC was performed with Etest for vancomycin (VA) and teicoplanin (TP). GRD was placed on Mueller Hinton agar containing 5% horse blood and inoculated with a 0.5 McFarland bacterial suspension. In MME, VA- and TP-Etest strips were placed on BHI-agar with a 2 McFarland bacterial suspension. The plates were read after 24 and 48 h incubation. The PAP-AUC method was performed by spot inoculation of BHI agar plates containing different concentrations of vancomycin and teicoplanin, respectively. After 24 and 48 h of incubation the number of cfu on the plates was counted. The cfu was plotted against the glycopeptide concentration and the area under the curve (AUC) was calculated and compared to the AUC of the Mu3 strain (*S. aureus* ATCC 700698).

Results

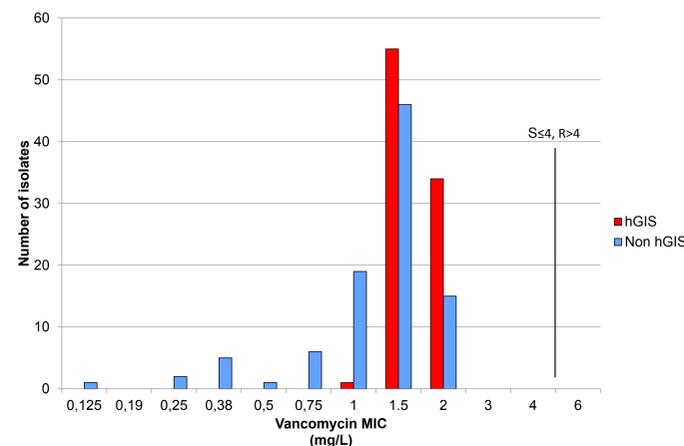


Figure 1: Vancomycin MIC distribution of the heterogeneously glycopeptide intermediate staphylococci (hGIS) and non-hGIS CoNS isolates. No CoNS isolates were vancomycin resistant according to the EUCAST breakpoint shown in the figure.

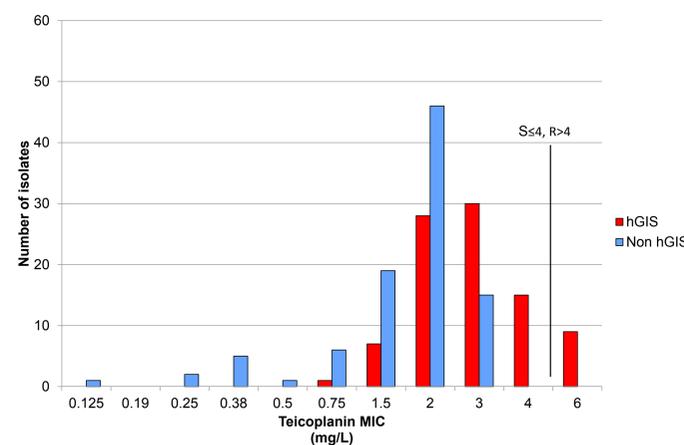


Figure 2: Teicoplanin MIC distribution of heterogeneously glycopeptide intermediate staphylococci (hGIS) and non-hGIS CoNS isolates. Resistance to teicoplanin was detected among 4.9% of the CoNS isolates using the EUCAST breakpoint shown in the figure.

Results

Table 1: Number and percentage of the CoNS isolates that were hGIS in the glycopeptide resistance detection test (GRD) and the macrodilution method with Etest (MME), respectively. All MME positive CoNS isolates were also positive in GRD.

hGIS according to	Number of hGIS isolates N=183	Percentage of hGIS isolates N=183
GRD	90	49.1
MME	20	10.9
Negative	93	50.8

Table 2: Number of CoNS isolates that were positive or negative in the population analysis profile-area under the curve (PAP-AUC), when using agar plates containing different concentrations of vancomycin, in relation to the results of the GRD and MME test, respectively.

	Positive PAP-AUC N=49	Negative PAP-AUC N=16
Positive GRD only	23	0
Positive MME only	0	0
Positive GRD and MME	20	0
Negative in both methods	6	16

Table 3: Number and percentage of the CoNS isolates that were correctly identified as hGIS according to the GRD and MME test, respectively.

	Positive PAP-AUC N=49 (%)
Positive GRD total	43 (87.7)
Positive MME total	20 (40.8)
Negative in both methods	6 (12.2)

Interpretation of data

The CoNS isolate was considered positive in the GRD if the GRD MIC was ≥ 8 mg/L for either vancomycin or teicoplanin or both after 48h incubation in 35° C according to the previous definitions for *S. aureus* [2].

The CoNS isolate was considered to be positive in the MME test if the MME MIC for both vancomycin and teicoplanin was ≥ 8 mg/L or ≥ 12 mg/L for teicoplanin only after 48 h incubation in 35° C, according to the *S. aureus* definitions [3].

The CoNS isolate was considered to be a hGIS in the PAP-AUC test if the ratio of the AUC of the test isolate and the AUC of the *S. aureus* Mu3, ATCC700698, on vancomycin plates, was ≥ 0.9 but < 1.3 and a glycopeptide intermediate staphylococci (GIS) if the ratio was ≥ 1.3 [4].

Conclusion

The GRD Etest correlated best with the results of the PAP-AUC method. The GRD is easy to use and the result is easy to interpretate. GRD can be a suitable method for detection of CoNS isolates with reduced susceptibility to glycopeptides in the clinical microbiology laboratory.

References

1. Wootton, M., et al., *A multicenter study evaluating the current strategies for isolating Staphylococcus aureus strains with reduced susceptibility to glycopeptides.* J Clin Microbiol, 2007. **45**(2): p. 329-32.
2. Yusof, A., et al., *Evaluation of a new Etest vancomycin-teicoplanin strip for detection of glycopeptide-intermediate Staphylococcus aureus (GISA), in particular, heterogeneous GISA.* J Clin Microbiol, 2008. **46**(9): p. 3042-7.
3. Walsh, T.R., et al., *Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides.* J Clin Microbiol, 2001. **39**(7): p. 2439-44.
4. Leonard, S.N., et al., *Evaluation of the Etest GRD for the detection of Staphylococcus aureus with reduced susceptibility to glycopeptides.* J Antimicrob Chemother, 2009. **63**(3): p. 489-92.