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Evaluation of the Etest GRD for the detection of *Staphylococcus aureus* with reduced susceptibility to glycopeptides

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***Objectives*: Continued glycopeptide-selective pressure has led to non-susceptible strains of *Staphylococcus aureus* including heterogeneously vancomycin-intermediate *S. aureus* (hVISA). The gold standard for identification of hVISA is the population analysis profile area under the curve ratio (PAP-AUC), though this method is time-consuming and labour-intensive. The objective of this study was to compare a new method for detection of hVISA, the Etest GRD, to PAP-AUC and to macro Etest.**

***Methods*: One hundred clinical hVISA and 50 clinical fully vancomycin-susceptible *S. aureus* (VSSA), confirmed by PAP-AUC, were evaluated. Microtitre and Etest MICs were determined by standard testing procedures on all isolates. Macro Etest was performed according to referenced procedures. The Etest GRD was tested using a 0.5 McFarland standard on Mueller–Hinton agar + 5% blood and read at 24 and 48 h. If either the vancomycin or the teicoplanin end of the GRD strip was ≥8 and the vancomycin Etest was ≤4, the isolate was considered hVISA.**

***Results*: Vancomycin MIC_{50}/MIC_{90} for hVISA and VSSA was 1.5/2 mg/L and 1/1.5 mg/L, respectively, by Etest and vancomycin MIC_{50}/MIC_{90} for hVISA and VSSA was 1/2 mg/L for both by microtitre; MIC values for hVISA being significantly higher ($P \leq 0.023$). At 24 h, the Etest GRD was 77% sensitive and 98% specific, and at 48 h, it was 93% sensitive and 82% specific compared with PAP-AUC. Macro Etest was 83% sensitive and 94% specific at 48 h.**

***Conclusions*: Etest GRD was simple to perform and may be feasible for clinical microbiology laboratories. This test may be useful for clinical detection of hVISA.**

Keywords: hVISA, hGISA, heteroresistance, MRSA

Introduction

The continued emergence of strains of *Staphylococcus aureus* with reduced susceptibility to glycopeptides, including heteroresistant strains such as heterogeneously vancomycin-intermediate *S. aureus* (hVISA), presents a clinical challenge. Infection with hVISA has been associated with high bacterial load infections, prolonged fever and bacteraemia, increased length of hospital stay and vancomycin treatment failure.[1–4] This is particularly concerning because these organisms generally go undetected in the clinical laboratory as they are considered vancomycin-susceptible by traditional MIC testing.[2,5] Due to this difficulty in detection, the prevalence of hVISA is difficult to estimate and ranges from ~2% to ~11%.[1,5–7] Our own study of hVISA from the Detroit Medical Center and sampling from the metropolitan area of Detroit, MI, demonstrated 8.3% hVISA for the period 2003–07 and also that the fraction of methicillin-resistant *S. aureus* (MRSA) strains that display this phenotype is increasing.[7] The 'gold standard' for detection of hVISA is the population analysis profile area under the curve ratio (PAP-AUC), but this method is time-consuming, labour-intensive and unsuitable for clinical laboratories. We evaluated a new method of detection, the Etest GRD, as well as the macrodilution method Etest[8] on 150 clinical isolates of MRSA characterized as either

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hVISA or not hVISA [vancomycin-susceptible *S. aureus* (VSSA)] by PAP-AUC.

Materials and methods

Bacterial strains

One hundred clinical hVISA, whose origins have been described previously,[7] and 50 clinical VSSA obtained from patients at the Detroit Medical Center were evaluated.

Susceptibility testing

Microtitre dilution methods and Etest using standard reference methods were performed on all isolates.[9]

PAP-AUC and macro Etest

All strains were characterized as either hVISA or not hVISA (VSSA) by PAP-AUC as described previously.[7] Briefly, 50 μL of a bacterial suspension at an inoculum of 10^{8-9} was plated on brain heart infusion agar (BHIA; Difco, Detroit, MI, USA) plates containing increasing concentrations of vancomycin (0, 0.5, 1, 2, 3, 4 and 8 mg/L) using an automated spiral plater (WASP, DW Scientific, West Yorkshire, UK) and read using a laser colony counter (ProtoCOL, Synoptics Limited, Frederick, MD, USA) after 48 h of incubation at 35°C. All PAP-AUC were performed in duplicate using Mu3 as a positive control. Interpretation of PAP-AUC was as follows: ratio of the AUC of the test isolate to Mu3 <0.9 was considered VSSA, ratio of the AUC of the test isolate to Mu3 ≥ 0.9 and <1.3 was considered hVISA and ratio of the AUC of the test isolate to Mu3 ≥ 1.3 was considered VISA. Macrodilution method Etests were done on all strains as described previously.[8]

Etest GRD

Evaluation with the Etest GRD was done according to the manufacturer's instructions. A bacterial suspension corresponding to a 0.5 McFarland standard was lawned on a Mueller–Hinton agar + 5% blood (MHB; Becton, Dickinson and Company, Sparks, MD, USA) plate and on a Mueller–Hinton agar (MHA; Difco) plate. A GRD strip consisting of a double-sided gradient with vancomycin and teicoplanin was then applied to the MHB plate and a standard vancomycin Etest was applied to the MHA plate. The standard vancomycin Etest was read and recorded after 18–24 h of incubation. The zone of the Etest GRD strip was also read, at complete inhibition of growth, at 24 and 48 h. The test isolate was considered positive for hVISA if the Etest GRD strip was ≥ 8 mg/L for either vancomycin or teicoplanin and the standard vancomycin Etest MIC was ≤ 4 mg/L.

Statistical analysis

Differences in vancomycin MIC between hVISA and VSSA were evaluated by the Mann–Whitney *U*-test using SPSS statistical software (Release 16.0, SPSS, Inc., Chicago, IL, USA). A *P* value of ≤ 0.05 was considered significant. Sensitivity and specificity analyses were performed to evaluate the performance of the GRD test versus PAP-AUC. Sensitivity analysis describes the fraction of correctly identified true positives (hVISA) by the Etest GRD strip, while specificity analysis describes the fraction of correctly identified negatives (VSSA).

Table 1. Etest vancomycin MIC distributions for hVISA and VSSA isolates

	Percentage of isolates with an MIC (mg/L) of					
	0.38	0.5	0.75	1	1.5	2
hVISA	0	0	2	8	41	49
VSSA	2	2	20	38	30	8

Results

The vancomycin MIC_{50}, MIC_{90} and range were 1.5, 2 and 0.75–2 mg/L for hVISA and 1, 1.5 and 0.38–2 mg/L for VSSA, respectively, by Etest; this difference in MIC distribution was statistically significant ($P < 0.001$). Most of the hVISA isolates (90%) had a vancomycin MIC >1 mg/L. Vancomycin MIC distributions for hVISA and VSSA isolates are displayed in Table 1. Overall, MIC by Etest tended to be slightly higher than microdilution MIC with a vancomycin MIC_{50}, MIC_{90} and range of 1, 2 and 0.5–2 mg/L for hVISA and 1, 2 and 0.5–2 mg/L for VSSA, respectively. Still, the difference in MIC distribution between hVISA and VSSA remained significant, with MIC values of hVISA tending to be higher, though not to the same degree as by Etest ($P = 0.023$). At 24 h, the Etest GRD was 77% sensitive and 98% specific. At 48 h, the Etest GRD displayed a sensitivity of 93% and a specificity of 82%. Of those tests that were positive at 24 h, 100% were positive at the teicoplanin end of the strip only. Examples of GRD strips at 48 h are shown in Figure 1. Macro Etest was 83% sensitive and 94% specific at 48 h.

Discussion

Vancomycin remains the mainstay of therapy for infections caused by MRSA. Unfortunately, strains of MRSA with reduced susceptibility to vancomycin, including hVISA, are increasing in prevalence, and infection with hVISA has been associated with vancomycin treatment failures.[1–4] Therefore, early detection of hVISA is of paramount importance. As demonstrated in this investigation, MIC values for hVISA by both microtitre and Etest methods are in the susceptible range, although there is a tendency for a higher percentage of hVISA strains detected at an MIC of 2 mg/L. This is similar to the findings reported previously.[7] Currently, there is no standardized method for detection of hVISA though PAP-AUC is considered the gold standard, a method too time-consuming and labour-intensive for a clinical laboratory. Other methods of detection of hVISA are available including BHIA plus 6 mg/L vancomycin (BHIA6V), MHA plus 5 mg/L teicoplanin (MHA5T) and macro Etest. In an investigation comparing these methods using PAP-AUC as the gold standard, both macro Etest and MHA5T performed similarly while BHIA6V displayed a poor sensitivity of 11%.[8]

We evaluated a new method for detection of hVISA that offers the possibility of being read at 24 h. While the sensitivity observed at 24 h (77%) was not as high as at 48 h, the high degree of specificity at 24 h (98%) indicates that a positive result at 24 h may have high significance. Positive and negative

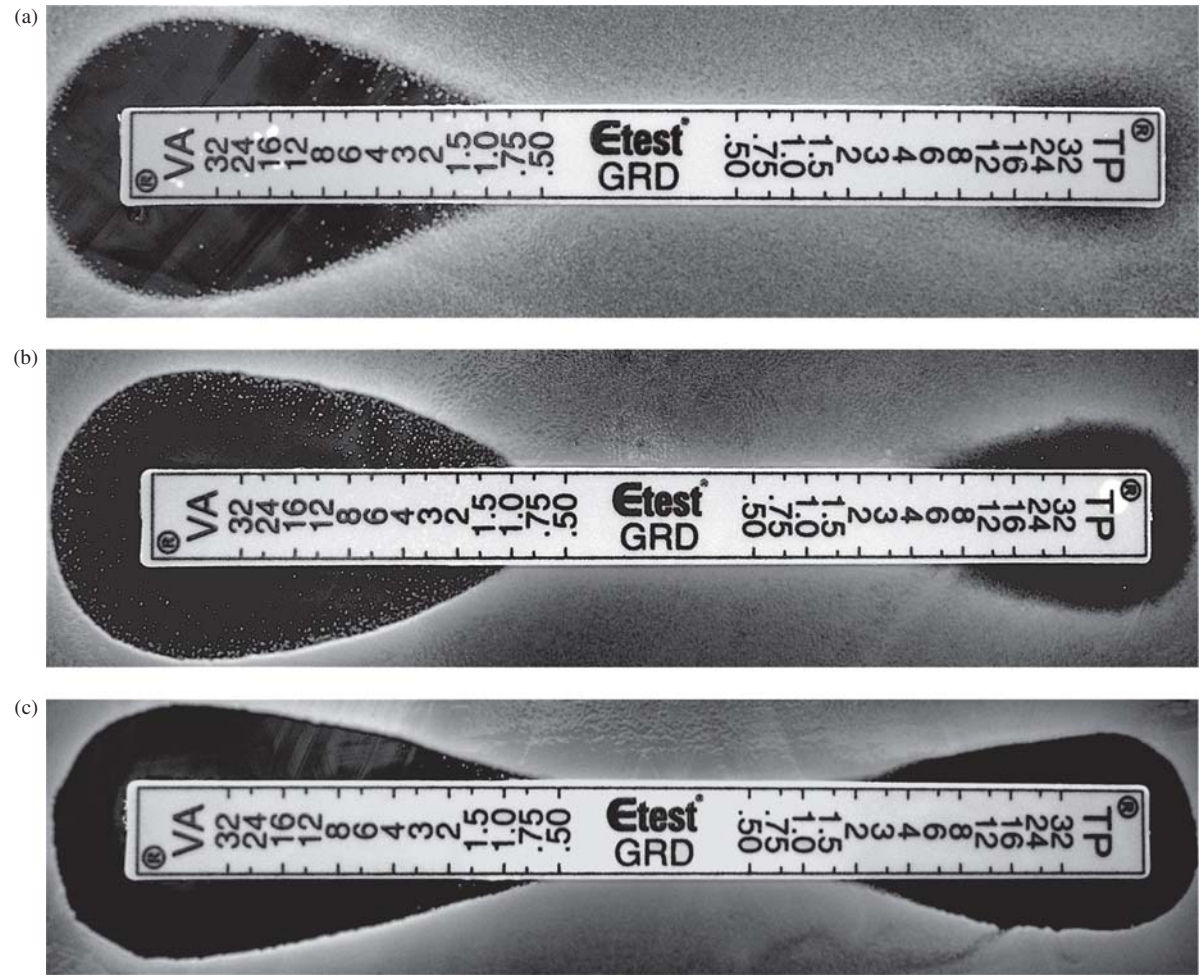

Figure 1. Etest GRD for hVISA Mu3 (a), a clinical hVISA with a positive Etest GRD (b) and a clinical VSSA with a negative Etest GRD (c).

predictive values, however, were not calculated due to the artificially high prevalence of hVISA in our cohort (100 of 150 strains, 67%) compared with the prevalence reported in the population (2–11%). We found the sensitivity of this test was improved to 93% by incubation for 48 h, underscoring the importance of reading the test not only at 24 h but also after a full 48 h of incubation, though this occurred at the expense of a lower specificity at this timepoint. This improvement in, and level of, sensitivity is consistent with recent data reported on the performance of the Etest GRD,[10] though the specificity reported at 48 h (95%) was higher than that reported by our investigation. The reason for this difference is not immediately clear as we used the same interpretive criteria, similar numbers of VSSA ($n = 50$ for our investigation and $n = 70$ for their investigation) and the same source for MHB plates (Becton, Dickinson and Company).

In conclusion, we evaluated a new Etest method for the detection of *S. aureus* with reduced susceptibility to glycopeptides. The test was simple to perform using standard media and inocula utilized in clinical microbiology laboratories. At 24 h, the test was sensitive at 77% with a very high specificity. The sensitivity was improved to >90% with 48 h of incubation, though the specificity declined at 48 h. Further research is warranted to determine the value of this test, particularly an evaluation of the positive and negative predictive values.

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Transparency declarations

None to declare.

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