ICAAC & IDSA 2008
D-2213

EVALUATION OF THE ACCURACY OF THE ETEST MACROMETHOD FOR DETECTION OF HETEROGENEOUS VANCOMYCIN-INTERMEDIATE *S. AUREUS* (hVISA) STRAINS

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ABSTRACT

Background: There are no standard methods or clear guidelines for hVISA detection. Population analysis profiling (PAP) is considered the gold standard, but this approach is labor-intensive and expensive. The Etest macromethod (MET) exhibited high sensitivity and specificity compared to PAP in previous studies, and has been used as screening or definitive test to report the prevalence of hVISA. We evaluated the accuracy of MET using various criteria.

Methods: 268 oxacillin-resistant *S. aureus* were selected from 1800 strains (9 USA hospitals) based on increased MIC to vancomycin (VAN; > 1 μg/ml) or daptomycin (DAP; > 0.5 μg/ml) or increased VAN MBC/MIC ratio (≥32). The susceptibility (S) to VAN and teicoplanin (TEI) was assessed by Etest using 2 McFarland inoculum density (MET). PAP was determined on strains with MET subpopulation detected at MIC level ≥4 μg/ml for VAN and ≥8 μg/ml for TEI, or ≥ 12 μg/ml for TEI alone, and also 9 randomly selected strains (1 per hospital) with TEI MIC < 4 μg/ml. Mu3, Mu50 and *S. aureus* ATCC 29213 were tested as controls and all results were within expected ranges.

Results: PAP was positive on 36 of 52 strains tested. Only 20 of 36 PAP positive strains met the most commonly used MET criteria for hVISA (TEI ≥12 or TEI ≥8 and VAN ≥8 μg/ml), and 3 strains that met this criteria were negative by PAP, including 2 strains with TEI at 12 and VAN at 3 μg/ml. The accuracies of the MET with various criteria are shown in the Table 1.

Conclusions: The accuracy of MET varies significantly with the criteria used for positivity, and the most frequently used criteria to define hVISA demonstrated low sensitivity, indicating that the prevalence of hVISA could be higher than currently appreciated.

INTRODUCTION

There are no standard methods or clear guidelines for detection of *Staphylococcus aureus* strains having hetero-resistance to vancomycin (hVISA). Population analysis profiling (PAP) is considered the "gold standard", but this approach is labor-intensive and costly.

The Etest macromethod (MET) exhibited high sensitivity and specificity compared to PAP in initial studies, and has been used as screening or as the definitive test to assess the prevalence of hVISA. The criteria most frequently used to characterize hVISA are those described by Wootton et al.[2007], e.g. MIC values ≥ 8 μg/ml for both vancomycin and teicoplanin or ≥12 μg/ml for teicoplanin alone after 48 hours of incubation at 35°C.

In this study, we evaluated the accuracy of MET by comparing the results of this method using various criteria with the results obtained by PAP analysis. We also evaluated the bactericidal activities of vancomycin and daptomycin in a large collection of methicillin-resistant *Staphylococcus aureus* (MRSA) strains from 9 major United States (USA) medical centers, each representing a different census region.

METHODS

Bacterial isolates and susceptibility testing:

As part of a large multicenter study, 1,800 MRSA strains from bloodstream infections were collected in 9 USA medical centers (one from each census regions). Each medical center was requested to randomly select 40 strains per year during the 2002-2006 period (total of 200 MRSA strains per center). All isolates were susceptibility tested against vancomycin, daptomycin and various other antimicrobial agents by reference broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI) M7-A7 [2006] guidelines. Mueller-Hinton broth adjusted to contain physiological levels of calcium (50 mg/L) was used when testing daptomycin. MBC tests were performed for vancomycin and daptomycin on 50% of strains (randomly selected) by plating the broth from the MIC endpoint well and from those four log2 dilutions above the MIC for each organism onto appropriate growth media.

Etest macromethod (MET):

A subset of isolates (n = 268) showing tolerance (MBC/MIC, ≥32) and/or increased MIC to vancomycin (>1 μg/ml) or increased MIC to daptomycin (>0.5 μg/ml) were susceptibility tested against vancomycin and teicoplanin by MET (AB BIODISK, Solna, Sweden) using both standard manufacturer recommendations (0.5 McFarland on Mueller-Hinton agar) and a high inoculum (2 McFarland) on brain-heart infusion (BHI) agar.

Population analysis (PAP):

PAP was determined on strains detected at MIC level ≥4 μg/ml for vancomycin and ≥8 μg/ml for teicoplanin, or ≥ 12 μg/ml for teicoplanin alone with MET method. Randomly selected strains (9) with low vancomycin and teicoplanin MIC values (<4 μg/ml) were also evaluated by PAP. Mu3, Mu50 and *S. aureus* ATCC 29213 were tested as controls and all results were within expected ranges.

Evaluation of the accuracy of the MET for detection of hVISA strains:

Sensitivity, specificity and predictive values of various criteria for characterization of hVISA, including those published by Wootton et al.[2007] (MIC values ≥ 8 μg/ml for both vancomycin and teicoplanin or ≥12 μg/ml for teicoplanin alone after 48 hours of incubation at 35 C), were evaluated by comparing the results of the MET and PAP.

RESULTS

- Among 268 MRSA strains tested by MET, 43 exhibited MIC ≥4 μg/ml for vancomycin and ≥8 μg/ml for teicoplanin, or ≥ 12 μg/ml for teicoplanin alone. These 43 strains plus 9 randomly selected strains with low vancomycin and teicoplanin MIC values (<4 μg/ml) were evaluated by PAP and 36 of those (69.2%) were characterized as hVISA by this method. The accuracy of the MET using various criteria for the detection of hVISA is shown on Table 1.
- The most commonly used MET criteria for hVISA (teicoplanin ≥12 or teicoplanin ≥8 and vancomycin ≥8 μg/ml) showed a sensitivity of only 55.6%, detecting only 20 of 36 PAP positive strains. Furthermore, 3 strains that met these criteria were negative by PAP, including 2 strains with teicoplanin at 12 and vancomycin at 3 μg/ml (Table 1).

Table 1. The accuracy of the Etest macromethod (MET) using various criteria for the detection of hVISA

Positive MET criteria (subpopulation detected at (MIC in μg/ml))	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
TEI ≥12 or TEI ≥8 and VAN ≥8[a]	55.6%	81.3%	87.0%	44.8%	63.5%
TEI ≥12 or TEI ≥8 and VAN ≥6	69.4%	68.8%	83.3%	50.0%	69.2%
TEI ≥12 or TEI ≥8 and VAN ≥4	97.2%	56.2%	83.3%	90.0%	84.6%
TEI ≥8 and VAN ≥4	97.2%	68.8%	87.5%	91.7%	88.5%

a. Most frequently used MET criteria to define hVISA

Abbreviations: TEI = teicoplanin and VAN = vancomycin.

- The highest sensitivity was obtained using a positive Etest macromethod criteria as the detection of subpopulation at vancomycin MIC ≥ 4 g/ml and teicoplanin MIC ≥ 8 g/ml (97.2%). If the detection of subpopulation with teicoplanin MIC ≥ 12 g/ml alone was included in the criteria, the specificity would decrease from 68.8 to 56.2% without any gain of sensitivity.
- Percentages of isolates displaying vancomycin tolerance (vancomycin MBC/MIC ratios ≥32 μg/ml) markedly varied by year between institutions and no clear trend in tolerance was apparent. Overall, 181 of 900 (20.1%) MRSA tested exhibited vancomycin tolerance (Figure 1), varying from 11.7% in 2004 to 27.8% in 2005 (Table 2). The occurrence of vancomycin tolerant MRSA varied from 10 to 43% among the medical centers evaluated (Table 3), and was particularly high among hVISA strains (47.2%).
- Daptomycin showed bactericidal activity against all strains tested. Daptomycin MBC/MIC ratio was 1 for 788 (87.6%) and 2 for 110 (12.2%) MRSA strains tested. The highest daptomycin MBC/MIC ratio value was 4 (only 2 [0.2%] strains; Table 2).

Figure 1. Summary of bactericidal activities of vancomycin and daptomycin tested against 900 MRSA strains.

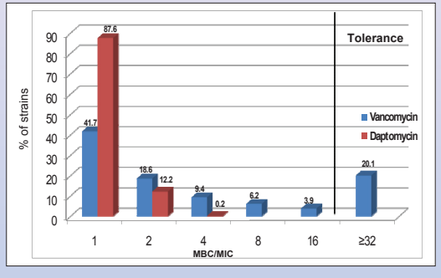

Table 2. Summary of bactericidal activities of vancomycin and daptomycin by year (all medical centers).

Year	No. of strains	No. of strains (%) with MBC/MIC ratio of: Vancomycin 1	2	4	8	16	≥32	Daptomycin 1	2	4	≥8
2002	180	73 (40.1)	33 (18.3)	21 (11.6)	12 (6.7)	6 (3.3)	35 (19.4)	153 (85.0)	27 (15.0)	0 (0.0)	0 (0.0)
2003	180	73 (40.1)	23 (12.8)	20 (11.1)	11 (6.1)	8 (4.4)	45 (25.0)	159 (88.3)	20 (11.1)	1 (0.6)	0 (0.0)
2004	180	88 (48.9)	38 (21.1)	15 (8.3)	13 (7.2)	5 (2.8)	21 (11.7)	159 (88.3)	20 (11.1)	1 (0.6)	0 (0.0)
2005	180	62 (34.4)	37 (20.1)	13 (7.2)	12 (6.7)	6 (3.3)	50 (27.8)	166 (92.2)	14 (7.8)	0 (0.0)	0 (0.0)
2006	180	79 (43.9)	37 (20.1)	16 (8.9)	8 (4.4)	10 (5.6)	30 (16.7)	151 (83.9)	29 (16.1)	0 (0.0)	0 (0.0)
Total	900	375 (41.7)	167 (18.6)	85 (9.4)	56 (6.2)	35 (3.9)	181 (20.1)	788 (87.6)	110 (12.2)	2 (0.2)	0 (0.0)

Table 3. Overall prevalence of vancomycin tolerant strains (MBC/MIC ≥32 g/ml) by medical center

Site no.	Tolerant strains (%)
1.	10.0
2.	23.0
3.	20.0
4.	13.0
5.	20.0
6.	10.0
7.	43.0
8.	13.0
9.	29.0

CONCLUSIONS

- The accuracy of the MET method varies significantly with the criteria used for positivity.
- The most frequently used criteria to define hVISA demonstrated low sensitivity, indicating that the prevalence of hVISA could be higher than currently appreciated.
- The MET criteria for detection of hVISA should be re-evaluated. Based on the results of this study, the criteria that correlated better with PAP analysis was teicoplanin ≥8 μg/ml and vancomycin ≥4 μg/ml, with sensitivity of 97.2% but a specificity of only 68.8%.
- The occurrence of vancomycin tolerant strains was generally high (20.1% overall), especially among hVISA strains (47.2%). However, no trend toward increasing prevalence of vancomycin tolerant or hVISA strains overtime was observed.
- In contrast to vancomycin, daptomycin was highly bactericidal against this large collection MRSA strains from bloodstream infections (2002-2006), including hVISA and vancomycin tolerant strains.

ACKNOWLEDGMENTS

The authors would like to thank the following investigators for kindly providing strains for the study: Paul D Fey, University of Nebraska Medical Center (Omaha, NE); Douglas Fish, University of Colorado Health Science Center (Denver, CO); Ajit Limaye, University of Washington (Seattle, WA); George Pankey, Ochsner Clinic Foundation (New Orleans, LA); James Rahal, New York Hospital Queens (New York, NY); Michael Rybak, Wayne State University (Detroit, MI); David Snydman, New England Medical Center (Boston, MA); Lisa L. Steed, Medical University of South Carolina (Charleston, SC); Ken Waites, University of Alabama at Birmingham (Birmingham, AL).

REFERENCES

1. Clinical and Laboratory Standards Institute (2006). M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition. Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2008). M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement. Wayne, PA: CLSI.
3. Fitzgibbon MM, Rossney AS, O'Connell B (2007). Investigation of reduced susceptibility to glycopeptides among methicillin-resistant *Staphylococcus aureus* isolates from patients in Ireland and evaluation of agar screening methods for detection of heterogeneously glycopeptide-intermediate *S. aureus*. J Clin Microbiol 45: 3263-3269.
4. Rybak MJ, Leonard SN, Rossi KL, Cheung CM, Sader HS, Jones RN (2008). Characterization of vancomycin heteroresistant *Staphylococcus aureus* (hVISA) from the Detroit Metropolitan area over a 22-year period (1986-2007). Antimicrob Agents Chemother 46: 2950-2954
5. Wootton M, MacGowan AP, Walsh TR, Howe RA (2007). A multicenter study evaluating the current strategies for isolating *Staphylococcus aureus* strains with reduced susceptibility to glycopeptides. J Clin Microbiol 45: 329-332.

This study was funded by Cubist Pharmaceuticals.