Effects of Storage on Vancomycin and Daptomycin MIC in Susceptible Blood Isolates of Methicillin-Resistant Staphylococcus aureus

Franziska Ludwig,a Becky Edwards,a* Timothy Lawes,b* and Ian M. Goulda

Medical Microbiology Department, Aberdeen Royal Infirmary, Aberdeen, United Kingdom,a and Raigmore Hospital, Inverness, United Kingdomb

By repeating Etests on 36 blood isolates of methicillin-resistant Staphylococcus aureus (MRSA) over 9 months, we explored the effects of isolate storage on vancomycin and daptomycin MICs. We identified overall declines in vancomycin and daptomycin MICs taken from the same isolates at 3-month intervals (P < 0.001). Declines of 1 doubling dilution were observed in 75% and 67% of isolates for vancomycin and daptomycin MICs, respectively. Effects of storage should be considered in evidence of MIC “creep.”

Decreasing effectiveness of vancomycin in the treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections has been linked to increasing vancomycin MIC within the susceptibility range (>2 mg/liter) (5). Subinhibitory vancomycin exposure may also be a risk factor for daptomycin heterogenous susceptibility (18, 22, 28). However, evidence of MIC “creep” is mixed (24), and the implications for treatment decisions of a vancomycin MIC that is high but still within the susceptibility range are uncertain (5). We previously demonstrated that detection of vancomycin and daptomycin MIC creep may be method dependent (7). In this study, we further explored the hypothesis that isolate storage may explain divergent results in investigations of susceptibility trends.

A prospective repeated-measure design involved susceptibility testing of MRSA bloodstream isolates (BSI) at the time of isolation and at 3-month intervals. A 9-month follow-up period was chosen on the basis of previous data suggesting that changes in susceptibility occurred within this time. Our sample included all nonreplicate MRSA BSI identified in adult, non-obstetric patients at Aberdeen Royal Infirmary (Scotland) between January and March 2011. Susceptibility testing at the time of isolation was by Etest performed in accordance with the manufacturer’s guidelines on Mueller-Hinton agar (Oxoid), and results were read blind in duplicate. Each isolate was then stored in a Cryobank storage container (Mast Diagnostics), which contained glycerol, peptones, sucrose, and saline, at −70°C. Isolates were recovered from storage at 3, 6, and 9 months and subcultured twice prior to repeat Etests. Assessors were blinded to previous MIC results.

Interobserver agreement was assessed by weighted Cohen’s kappa (κ). Friedman tests were used to test for overall difference in MICs across repeated readings, with post hoc analyses by Wilcoxon signed-rank tests with Bonferroni adjustment (α = 0.0083). Variation in trends was investigated by independent-sample t test to assess differences in means of slopes for isolates grouped by “high” (>median) or “low” (<median) baseline MICs. All analyses were repeated with MICs from Etest converted to doubling dilutions by rounding up intermediate increments.

To evaluate the potential for systematic bias arising from differences in storage of isolates before susceptibility testing in stud-

<table>
<thead>
<tr>
<th>Drug and time of testing</th>
<th>Mean MIC (95% CI)a</th>
<th>Modal MIC</th>
<th>MIC range</th>
<th>No. (%) of isolates with MIC &gt;baseline median</th>
<th>MIC50</th>
<th>MIC90</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vancomycin</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>1.21 (0.63–1.79)</td>
<td>1.50</td>
<td>0.38–1.50</td>
<td>17 (47)</td>
<td>1</td>
<td>1.50</td>
</tr>
<tr>
<td>3 mo</td>
<td>0.84 (0.15–1.54)</td>
<td>1.00</td>
<td>0.00–1.50</td>
<td>5 (14)</td>
<td>0.75</td>
<td>1.50</td>
</tr>
<tr>
<td>6 mo</td>
<td>0.77 (0.38–1.16)</td>
<td>0.75</td>
<td>0.38–1.00</td>
<td>0 (0)</td>
<td>0.75</td>
<td>1.00</td>
</tr>
<tr>
<td>9 mo</td>
<td>0.65 (0.25–1.04)</td>
<td>0.75</td>
<td>0.25–1.00</td>
<td>0 (0)</td>
<td>0.75</td>
<td>0.75</td>
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<tr>
<td><strong>Daptomycin</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.23 (0.00–0.47)</td>
<td>0.25</td>
<td>0.09–0.75</td>
<td>18 (50)</td>
<td>0.19</td>
<td>0.38</td>
</tr>
<tr>
<td>3 mo</td>
<td>0.22 (0.01–0.43)</td>
<td>0.19</td>
<td>0.00–0.50</td>
<td>15 (42)</td>
<td>0.19</td>
<td>0.38</td>
</tr>
<tr>
<td>6 mo</td>
<td>0.24 (0.03–0.44)</td>
<td>0.125</td>
<td>0.00–0.50</td>
<td>14 (39)</td>
<td>0.19</td>
<td>0.38</td>
</tr>
<tr>
<td>9 mo</td>
<td>0.08 (0.04–0.20)</td>
<td>0.125</td>
<td>0.06–0.25</td>
<td>1 (3)</td>
<td>0.125</td>
<td>0.19</td>
</tr>
</tbody>
</table>

a MIC units are mg/liter.
b There were 36 isolates for each test time.
c CI, confidence interval.

October 2012 Volume 50 Number 10 Journal of Clinical Microbiology p. 3383–3387 jcm.asm.org
ies reporting MIC creep, we searched Medline and PubMed databases for original articles published after 2000 (all languages) using the following search terms: (i) “MIC creep” or “resistance trend*” or “susceptibility trend*” or “decreas* susceptibility,” (ii) “vancomycin” or “glycopeptide,” and (iii) “Staphylococcus aureus” or “MRSA” or “Gram-positive.” Reference lists of original articles were also searched. We excluded case studies, studies of treatment effects, cross-sectional designs, susceptibility testing by automated agar dilution methods, and reports of vancomycin resistance without reference to actual MICs.

Thirty-six MRSA BSI were identified between January and March 2011. All duplicate readings were within one Etest increment with “excellent” interobserver agreement (κ = 0.980). There were significant differences in vancomycin and daptomycin (P < 0.001) MICs from the same isolates measured at 3-month intervals (Table 1 and Fig. 1). Findings were unchanged by use of doubling dilutions. Declines of ≥1 doubling dilution and ≥2 doubling dilutions were observed in 67% to 75% and 11% of isolates, respectively. Post hoc comparisons (see the supplemental material) found that declines in vancomycin and daptomycin MICs occurred within 3 months and after 6 months, respectively (Fig. 2). The average decline in vancomycin MIC was significantly steeper for isolates for which the MIC was higher (>1.0 mg/liter) at baseline (−0.08 mg/liter · 3 months⁻¹ versus −0.04 mg/liter · 3 months⁻¹; P = 0.002). Trends in daptomycin susceptibility were not related to baseline MIC.

A literature search identified 376 papers. After exclusions, 16 were relevant. Review of references in these papers identified 3 other studies. Both studies performed at the time of isolation suggested evidence of vancomycin MIC creep; however, creep was less consistently found by Etest or broth microdilution (BMD) after storage, although heterogeneity in study population and size was noted (see the supplemental material) (1, 2, 6, 11, 13, 14, 20, 23, 26, 29, 35, 36).

This prospective longitudinal study found evidence that, in daptomycin- and vancomycin-susceptible blood isolates, MIC results from Etest were inversely related to the duration of isolate storage prior to susceptibility testing. A review of studies describing vancomycin MIC creep suggested that failure to account for storage may introduce systematic errors.

Our study had a number of limitations. The small sample size may have caused under- or overestimation of the effects of storage. Consistency in the direction and the size of changes in MIC suggested that trends were not explained by lack of precision in testing method. Finally, we did not define MRSA strains of isolates, which may be important for generalizability. We have previously reported dominance of multilocus sequence type (MLST) clonal complexes 22 and 30 in MRSA blood isolates in the same...

FIG 1 Vancomycin (a) and daptomycin (b) MICs at baseline and after 3, 6, and 9 months of storage for 36 isolates.
population and differences in MICs after storage irrespective of strain (7).

Although a number of studies have found vancomycin and daptomycin MICs and MIC creep to be method dependent (19, 25, 27, 31), we are not aware of any detailing the effects of storage of MRSA isolates on MIC in susceptible isolates. van Griethuysen et al. (30) found a relationship between loss of the mecA gene in MRSA isolates and duration of storage, suggesting that mecA-negative cells may have a survival advantage or that loss of mecA occurs during storage; such deletions have been associated with vancomycin-intermediate S. aureus (VISA) (15). A larger study found no evidence of loss of resistance but suggested that variable genetic stability of MRSA strains and freeze-thaw strains used for cryostocking may be important to the reliability of susceptibility testing in frozen isolates (33). The vancomycin-resistant phenotype is known to be unstable in the absence of selective pressure (15), with vancomycin-susceptible revertant mutations observed after repeated passages on nonselective media (3). Genetic instability may impact phenotypic characteristics correlated with low-level vancomycin resistance, including cell wall thickness (4, 12, 15).

Differences in results from prospective and retrospective susceptibility testing raise concerns around interpretation of studies conducted on isolates after extended periods of storage. A recent meta-analysis finding association between high vancomycin MIC within the susceptibility range and outcomes in MRSA infections accounted for susceptibility testing method but not storage of isolates (32). There is a need to clarify the relevance to treatment decisions of high vancomycin MICs within the susceptibility range (5); this clarification should also be informed by baseline health, site (34) and severity of infection, source elimination, patient and population antibiotic exposures (9, 21), MRSA strain (15), and regional factors (8, 17). The performance of routinely used methods for detecting low-level glycopeptide resistance has been questioned, particularly given the apparent instability of resistant phenotypes in vitro (10, 16), and our study suggested that the effects of storage should be included in standardization of susceptibility testing (15).

Interpretation of evidence of MIC creep and effects on treatment efficacy should account for susceptibility testing methods used and storage prior to testing. We suggest that the clinical relevance of findings will be optimized when MIC results are from susceptibility testing around the time of isolation. This may be particularly relevant to surveillance programs receiving stored isolates.
ACKNOWLEDGMENTS
We are grateful to the staff in the diagnostic laboratory and media departments of Aberdeen Royal Infirmary for their help. No direct funding was attached to this study. I. M. Gould has received personal and grant financial support from companies manufacturing diagnostics and therapeutics for MRSA. B. Edwards has received grant financial support from companies manufacturing diagnostics and therapeutics for MRSA. T. Lawes and F. Ludwig have no conflicts of interest to declare.

This work was performed at the Medical Microbiology Department, Aberdeen Royal Infirmary, Aberdeen, United Kingdom.

REFERENCES

FIG 2 Changes in vancomycin (a) daptomycin (b) MICs by time period as measured by doubling dilutions and Etest increments and trend in mean MICs (Etest) relative to baseline MICs. CI, confidence interval. *, significant difference between data points by related-sample Wilcoxon signed-rank test.
Effects of Storage on Susceptibility of MRSA Isolates


