Activities of Vancomycin, Ceftaroline, and Mupirocin against *Staphylococcus aureus* Isolates Collected in a 2011 National Surveillance Study in the United States

Sandra S. Richtera, Daniel J. Diekemab, Kristopher P. Heilmannb, Cassie L. Dohrn, Emily K. Crispell, Fathollah Riahib, Jennifer S. McDanell, Sarah W. Satolac, Gary V. Doernb

Cleveland Clinic, Cleveland, Ohio, USA; University of Iowa Carver College of Medicine, Iowa City, Iowa, USA; Emory University, Atlanta, Georgia, USA

Forty-two medical centers from throughout the United States participating in a longitudinal surveillance program were asked to submit 100 consecutive *Staphylococcus aureus* isolates during July to December 2011. Susceptibility testing using CLSI broth microdilution and meca detection by PCR analysis was performed on the 4,131 isolates collected. Methods employing Etest glycopeptide resistance detection (GRD; bioMérieux) and brain heart infusion agar containing 4 mg/mL vancomycin (BHIV) were used to screen methicillin-resistant *S. aureus* (MRSA) isolates for heterogeneous intermediate-level resistance to vancomycin (hVISA). Isolates with positive hVISA screen results were confirmed by population analysis profiling-area under the curve (PAP-AUC) determinations. The genetic relatedness of hVISA, ceftaroline-nonsusceptible, or high-level (HL) mupirocin resistance MRSA isolates was assessed by pulsed-field gel electrophoresis (PFGE). Among 2,093 MRSA isolates, the hVISA screen results were positive with 47 isolates by Etest GRD and 30 isolates by BHIV agar screen. Twenty-five of the GRD- or BHIV screen-positive isolates were confirmed as hVISA by PAP-AUC testing. Results of the current study were compared to results obtained from prior surveillance performed in 2009. The prevalence of hVISA among MRSA isolates was higher in 2011 than in 2009 (1.2% versus 0.4%, P = 0.003), especially for isolates with a vancomycin MIC of 2 (45.4% versus 14.3%, P = 0.01). The overall rate of ceftaroline susceptibility in the current study was 99.4% (one hVISA isolate had an intermediate ceftaroline MIC). HL mupirocin resistance increased from 2.2% in 2009 to 3.2% in 2011 (P = 0.006). Although overall rates of hVISA and HL mupirocin resistance are low, they have increased since 2009.

*Staphylococcus aureus* is a major pathogen in the health care setting. According to 2009 to 2010 National Healthcare Safety Network data, *S. aureus* is the most common etiology of surgical-site infections and ventilator-associated pneumonia, causing 30% and 24% of cases, respectively (1). Only coagulase-negative staphylococci cause more central line-associated bloodstream infections than *S. aureus* (1).

Testing to detect nasal carriage of *S. aureus* is commonly performed prior to elective cardiothoracic surgery and in intensive care unit (ICU) patients. Positive results are usually followed by contact isolation and/or decolonization with intranasal mupirocin to prevent methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) infections (2, 3). Strains with high-level (HL) mupirocin resistance (MIC ≥ 512 µg/mL) have been associated with *mupA* or *mupB* genes encoding altered tRNA synthetases (4, 5). Testing for mupirocin resistance is not commonly performed in clinical laboratories, and most published data come from single-center studies.

Vancomycin continues to be the drug of choice for treating MRSA infections (6, 7). Fortunately, nonsusceptible strains, i.e., strains with vancomycin MICs above the CLSI susceptibility breakpoint (≥ 2 µg/mL), are rare (8, 9). There are concerns regarding the efficacy of vancomycin therapy for *S. aureus* isolates with heterogeneous intermediate-level resistance to vancomycin (hVISA) that appear susceptible when tested by standard MIC methods (10). The technical difficulty of performing the reference standard test for hVISA, population analysis profiling with area under the curve (PAP-AUC), limits its use to the research setting (11). Commercial “research use only” tests for hVISA detection are not performed routinely in clinical laboratories due to a low positive predictive value (12, 13) and the uncertain clinical implication of hVISA (14, 15). A meta-analysis demonstrated higher glycopeptide treatment failure rates for hVISA but no differences in mortality between patients with hVISA and vancomycin-susceptible MRSA infections (16).

The primary objective of this multicenter study was to determine rates of antimicrobial resistance among clinical isolates of *S. aureus* collected in a United States surveillance program during 2011 and to assess changes since 2009 (17). Monitoring of ceftaroline susceptibility was of particular interest to determine if the anti-MRSA activity of this novel β-lactam would persist following FDA approval in 2010. Methods to detect hVISA and HL mupirocin resistance were included in both study periods (12, 17). In the current study, two different hVISA screening methods, the brain heart infusion agar (BHI) method with vancomycin and the Etest glycopeptide resistance detection (GRD, bioMérieux) method, were compared with PAP-AUC as the reference method. The epidemiology of hVISA strains, ceftaroline-nonsusceptible strains, and strains with HL mupirocin resistance was examined.

(This work was presented in part as an abstract at the 52nd...
Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 10 September 2012 [18].

MATERIALS AND METHODS

Bacterial isolates. One hundred clinically significant S. aureus isolates were requested from medical centers participating in a longitudinal surveillance program tracking antimicrobial resistance in the United States. The S. aureus isolates were obtained from different patients during July to December 2011 and then sent to the University of Iowa central laboratory for testing. Details of the surveillance program have been previously published (17). Results of the current study were compared to results obtained from prior surveillance performed in 2009 (17).

Antimicrobial susceptibility testing. Susceptibility to 12 antimicrobial agents was assessed with the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (19, 20). The endpoint for linezolid MICs was read at 90% inhibition. FDA break points were applied for tigecycline. Staphylococcus aureus ATCC 29213, S. aureus ATCC BAA-977, and S. aureus ATCC BAA1708 were tested for quality control. Screening for mecA was performed on all isolates by PCR using modifications (17) of previously published methods (21). Vancomycin MICs were also determined by standard Etest for MRSA isolates (inoculation of Mueller-Hinton agar and reading after 24 h of incubation) in conjunction with the Etest GRD described below. Two methods were used to screen MRSA for hVISA.

Etest GRD. The Etest GRD was used to screen MRSA isolates for hVISA. Mueller-Hinton agar containing 5% sheep blood was inoculated with a 0.5 McFarland suspension of overnight growth followed by GRD strip placement. After 24 and 48 h of incubation in ambient air at 35°C, GRD tests were read. A positive GRD result for hVISA was a vancomycin or teicoplanin MIC of ≥8 μg/ml at 24 or 48 h.

BHIV screen. Screening of MRSA for hVISA was performed using a previously published BHI vancomycin (BHIV) screening agar method (22). The BHIV agar was prepared in-house by supplementing BHI agar (Becton, Dickinson and Company) with 4 μg/ml vancomycin (Sigma) and 16 g/liter pancreatic digest of casein (Becton, Dickinson and Company). The BHIV plates were used to screen MRSA isolates for hVISA by inoculation with four 10-μl drops of a 0.5 McFarland suspension. After 24 and 48 h of incubation at 35°C, plates were examined for growth. A positive BHIV screen result for hVISA was defined as growth of two or more colonies from one droplet.

PAP-AUC confirmation of hVISA. PAP-AUC testing was performed as previously described (23, 24) with modifications (12, 22) on isolates with a positive BHIV or GRD test result. A 100-μl volume of serial dilutions of 10−6 to 10−2 was spiral plated onto BHI agar without vancomycin, and four 10-μl drops of serial dilutions of 10−6 to 10−5 were plated onto BHI agar plates with vancomycin concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, and 8.0 μg/ml. After 24 and 48 h of incubation at 35°C, log10 values of CFU were plotted against vancomycin concentrations. Isolates with an AUC ratio (isolate AUC divided by hVISA strain Mu3 AUC) of 0.90 or greater were defined as hVISA (11).

PFGE. The genetic relatedness of mecA-positive isolates that were also HL mupirocin resistant, ceftaroline nonsusceptible, or hVISA was assessed using standard PFGE methods (25). After digestion of DNA with Smal (Sigma-Aldrich, St. Louis, MO), restriction fragments were separated by electrophoresis on a Chef DRII apparatus (Bio-Rad Laboratories, Hercules, CA) and analyzed using Bionumerics software (Applied Maths, Kortrijk, Belgium). Dendrogram construction used the unweighted-pair group method. Isolates with a similarity coefficient ≥0.8 were assigned to the same PFGE type. This analysis included comparison to PFGE type strains USA100 to USA1200 (26).

Statistical analysis. Differences in resistance rates and demographic characteristics were assessed for statistical significance using Fisher’s exact test. Two-tailed P values were determined, and values ≤0.05 were considered significant.

RESULTS

A total of 4,131 S. aureus clinical isolates were collected from 42 U.S. medical centers in 2011. The specimen sources included wounds or abscesses (51%), blood (25%), the lower respiratory tract (12%), tissue (5%), and other normally sterile sites (7%). Data corresponding to patient age and gender and specimen sources in 2011 were similar to those associated with isolates collected in 2009 (17).

Table 1 depicts the MIC distributions for the 4,131 S. aureus isolates collected in 2011. A comparison of these distributions for MRSA versus MSSA is found in Table 2. The agents demonstrating the largest differences between MRSA and MSSA in rates of susceptibility were erythromycin (<10% versus 61%) and levofloxacin (36% versus 91%). Ceftaroline, tigecycline, daptomycin, linezolid, vancomycin, tetracycline, and trimethoprim-sulfamethoxazole (TMP-SMX) demonstrated high (>95%) rates of MRSA and MSSA susceptibility.

The 23 isolates with elevated cefazoline MICs (2 μg/ml) were from patients in every geographic region (14 different centers). These cefazoline-intermediate isolates were all MRSA and assigned to 12 different PFGE types: USA100 (n = 7), USA200 (n = 21).
TABLE 2 MIC distributions for agents against methicillin-resistant and methicillin-susceptible S. aureus

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MRSA* (n = 2,093)</th>
<th>MSSA* (n = 2,038)</th>
<th>S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC range (µg/ml)</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>0.06 to 2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>≤0.06 to &gt;32</td>
<td>0.12</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>≤0.06 to 2</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.12 to &gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤0.12 to &gt;64</td>
<td>4</td>
<td>64</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.25 to 8</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0.25 to &gt;16</td>
<td>&gt;16</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤0.03 to 1</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>≤0.06 to &gt;64</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>≤0.03 to &gt;64</td>
<td>0.06</td>
<td>0.12</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>≤0.25 to 2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

a Methicillin-resistant S. aureus (MRSA) isolates were identified by a positive mecA PCR result.

b Methicillin-susceptible S. aureus (MSSA) isolates were identified by a negative mecA PCR result.

c Susceptibility (S) was determined using CLSI interpretive criteria, with the exceptions noted below.

d The FDA interpretive criterion for susceptibility is ≤0.5 µg/ml.

2), USA300 (n = 1), 3 other PFGE types with two or three isolates (n = 7), and 6 PFGE types with one isolate. Six centers submitted multiple (two to four) isolates with reduced ceftaroline susceptibility, but only one center submitted multiple isolates of the same PFGE type (two USA100 isolates).

The in vitro resistance rates for most agents showed little change since 2009. The proportion of MRSA isolates was slightly lower in 2011 (51%) than in 2009 (53%) (P = 0.01). The largest proportional change in resistance rate was seen for high-level (HL) mupirocin resistance. Although HL mupirocin resistance is still uncommon, the HL mupirocin resistance rate increased by approximately 40% both overall (from 2.2% in 2009 to 3.2% in 2011, P = 0.006) and among MRSA isolates only (from 2.8% in 2009 to 4.0% in 2011, P = 0.04). The PFGE analysis of the 84 MRSA isolates with HL mupirocin resistance from 34 centers showed that 50% were USA300 (n = 42), 9.5% were USA100 (n = 8), and 40% were 18 different PFGE types (n = 34) with 1 to 3 isolates assigned to each. Twelve of the 34 centers submitted multiple MRSA isolates with HL mupirocin resistance of the same PFGE type: two or three USA300 isolates from nine centers, five USA300 isolates from one center, and two non-USA PFGE type isolates from two centers.

Screening of the 2,093 MRSA isolates for hVISA yielded positive results for 47 isolates by Etest GRD (teicoplanin MIC of ≥8 µg/ml). The BHIV agar screen for hVISA had positive results for 30 isolates. Both screening tests were positive for 17 isolates, and only one test (GRD or BHIV) was positive for 43 isolates. Twenty-five of the 60 screen-positive isolates were confirmed to be hVISA by PAP-AUC testing. This represents a higher prevalence of hVISA among MRSA in 2011 (1.2%) than in the 2009 surveillance period (0.4%, P = 0.003), especially among MRSA isolates with a vancomycin MIC of 2 (45.4% versus 14.3%, P = 0.01) (12).

Fourteen (56%) of the 25 hVISA isolates were detected by the Etest GRD and BHIV screen agar (Table 3). The GRD screen detected an additional 10 isolates, whereas the BHIV screen agar detected only one more isolate. Twelve hVISA isolates were detected by the GRD screen after 24 h of incubation. The other 12 hVISA isolates detected by the GRD screen required 48 h of incubation. The positive predictive values for the two screening tests were similar (51% for GRD and 50% for BHIV agar). With one exception, all of the isolates with positive hVISA screen and negative PAP-AUC results had a vancomycin MIC of 1 µg/ml. The vancomycin MIC for a majority (56%) of the confirmed hVISA isolates was also 1 µg/ml. Rates of resistance (including intermediate-level resistance) to most agents for the 25 hVISA isolates were higher than those of the 2,046 GRD screen-negative MRSA isolates: for erythromycin, 96.0% versus 90.4%; for clindamycin, 64% versus 23.5%; for levofloxacin, 92.0% versus 63.5%; for trimethoprim-sulfamethoxazole, 8.0% versus 2.8%; for tetracycline, 16% versus 3.8%; for tigecycline, 8.0% versus 0.6%; for linezolid, 0% versus 0.01%; for daptomycin, 4% versus 0.1%; and for ceftaroline, 4% versus 1%. The only hVISA isolate with an elevated ceftaroline MIC (2 µg/ml) was also tigecycline nonsusceptible (MIC, 1 µg/ml).

The proportion of hVISA isolates that were recovered from wounds and abscesses (32%) was smaller than the proportion of GRD-negative isolates (55%, P = 0.03). The proportions of GRD screen-negative and hVISA isolates from blood cultures were similar (24% versus 40%, P = 0.09). Twenty-eight percent of the hVISA isolates were from tissue (n = 2), lower respiratory tract (n = 2), or normally sterile body fluid (not cerebrospinal fluid).

TABLE 3 Comparison of Etest GRD and BHIV screen agar for the detection of 25 hVISA isolates

<table>
<thead>
<tr>
<th>Vancomycin MIC (µg/ml)</th>
<th>No. (%) of MRSA isolates</th>
<th>Etest GRD positive (n = 47)</th>
<th>BHIV agar positive (n = 30)</th>
<th>hVISA* (n = 25) initially detected by:</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1 (0.05)</td>
<td>hVISA*</td>
<td>Not hVISA</td>
<td>BH + GRD</td>
</tr>
<tr>
<td>0.5</td>
<td>208 (9.9)</td>
<td>hVISA*</td>
<td>Not hVISA</td>
<td>BHI only</td>
</tr>
<tr>
<td>1</td>
<td>1,862 (89)</td>
<td>14 hVISA</td>
<td>5 hVISA</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>22 (1.1)</td>
<td>10 hVISA</td>
<td>0 hVISA</td>
<td>9</td>
</tr>
</tbody>
</table>

| Total                  | 2,093                    | 24 hVISA                  | 15 hVISA                   | 14                                       |

a Screening for hVISA was performed on MRSA (mecA-positive) isolates only.
b Determined by CLSI broth microdilution method.
c PAP-AUC ratio ≥ 0.90.
d Three isolates with negative PAP-AUC results were positive by BHIV and GRD screen tests.
The 25 hVISA isolates were received from 17 medical centers in eight geographic regions of the United States. The PFGE analysis of hVISA isolates revealed 16 different PFGE types. Five hVISA isolates had USA300 PFGE patterns, and six isolates were USA100. The other 14 hVISA isolates had unique PFGE profiles. Five centers submitted multiple hVISA isolates (two hVISA isolates from two centers, three hVISA isolates from one center, and four hVISA isolates from one center). Only two centers submitted multiple hVISA isolates with the same PFGE type (USA300).

A comparison of the vancomycin MICs determined by broth microdilution to those determined by standard Etest for MRSA is shown in Table 4. The majority (61%) of isolates had MICs of 1.5 μg/ml. The other 14 hVISA isolates had unique PFGE profiles. Five centers submitted multiple hVISA isolates (two hVISA isolates from two centers, three hVISA isolates from one center, and four hVISA isolates from one center). Only two centers submitted multiple hVISA isolates with the same PFGE type (USA300).

A recent study demonstrated high variability when the results of vancomycin MIC determinations derived from automated systems were compared to those obtained by broth microdilution, with the Phoenix system having the greatest tendency to underestimate a vancomycin MIC of 2 μg/ml (27). However, the results of most automated systems were within the 1 log₂ dilution variability expected with an MIC test.

It has been suggested that clinical laboratories should determine vancomycin MICs using Etest (27, 28). The Etest is more expensive and labor-intensive than automated systems, and our data show variability for this method as well. The most common Etest MIC of 1.5 μg/ml occurred for 67% of the MRSA isolates and usually correlated with broth microdilution MICs of 1 but also represented 36% of the isolates with a broth microdilution MIC of 2 (Table 4). A recent review (7) affirmed Infectious Diseases Society of America (IDSA) guidance to use clinical response for deciding whether to discontinue vancomycin therapy for patients with MRSA isolates with a MIC of 2 μg/ml (6).

The detection of higher rates of HL mupirocin resistance (3.2% overall, 4% of MRSA isolates) in 2011 was not unexpected. In 2005 to 2006, the CDC detected HL mupirocin resistance in 2.3% of invasive MRSA using a lower break point of >128 (29). A Canadian surveillance program observed an increase in HL mupirocin resistance among MRSA isolates from 1995 to 1999 (1.6%) to 2000 to 2004 (7.0%) (30). A single-center surgical intensive care unit (SICU) study found 13.2% of 302 MRSA isolates from nares to be HL mupirocin resistant (31). Screening of 1,089 patients with community-onset cutaneous infections detected only 2.1% colonized with an S. aureus isolate with HL mupirocin resistance (32).

The prevalence of isolates with HL mupirocin resistance that were USA300 (50%) and USA100 (9.5%) did not exceed the overall distribution of these PFGE types among all MRSA isolates in 2009 (17). The detection of a new genetic variant, mupB, in strains with HL mupirocin resistance is interesting, and studies to determine prevalence are needed (4). A recent randomized prospective study demonstrating the greatest reduction in infection rates among those universally decolonized with intranasal mupirocin suggests that the use of this drug and resistant populations are likely to increase (33).

The continued nearly uniform activity of ceftaroline (99.4% susceptibility) since FDA approval is encouraging. A dominant clone was not detected among the MRSA isolates with intermediate-level resistance to ceftaroline. Good activity of ceftaroline (MIC ≤ 1 μg/ml) was noted for 96% of hVISA and all daptomycin-nonsusceptible isolates. The only ceftaroline-resistant isolates (MICs of ≥4 μg/ml) that have been described previously in the literature were from four patients in Athens, Greece, during 2008 and 2009 (34). These four isolates were represented by a single clone (34).

In conclusion, the primary changes noted in U.S. surveillance during 2011 were increased rates of HL mupirocin resistance and hVISA compared to 2009. More hVISA isolates were detected by the Etest GRD than by the BHIV agar method. Both hVISA screening methods require PAP-AUC confirmation. Cefartole remained active against MRSA and hVISA isolates. Continued surveillance for resistant S. aureus populations is important to direct prevention and treatment strategies.

Table 4: Comparison of vancomycin MICs determined by Etest and the CLSI broth microdilution method for 2,093 MRSA isolates

<table>
<thead>
<tr>
<th>Vancomycin MIC (μg/ml)</th>
<th>No. of MRSA isolates</th>
<th>No. isolates with indicated Etest vancomycin MIC (μg/ml)</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
<th>1.5</th>
<th>2.0</th>
<th>3.0</th>
<th>4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥0.5</td>
<td></td>
<td></td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>208</td>
<td></td>
<td>9</td>
<td>18</td>
<td>72</td>
<td>106</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1,862</td>
<td></td>
<td>7</td>
<td>35</td>
<td>391</td>
<td>1,278</td>
<td>150</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>10</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>2,093</td>
<td></td>
<td>17</td>
<td>53</td>
<td>464</td>
<td>1,392</td>
<td>163</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

* Determined by CLSI broth microdilution method.
REFERENCES


