In Vitro Susceptibilities and Molecular Analysis of Vancomycin-Intermediate and Vancomycin-Resistant Staphylococcus aureus Isolates

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Background. There is increasing frequency of vancomycin-intermediate and -resistant Staphylococcus aureus (VISA and VRSA) isolates identified in clinical practice. There are limited reports evaluating susceptibility patterns and molecular characteristics of these strains.

Methods. Laboratory analysis was performed on 13 VRSA and 33 VISA isolates, including susceptibility testing by broth microdilution, detection of Panton-Valentine leukocidin (PVL) genes, arginine catabolic mobile element (ACME), and staphylococcal cassette chromosome mec typing using polymerase chain reaction. Strain typing using pulsed-field gel electrophoresis (PFGE) was performed on VRSA isolates.

Results. Telavancin, linezolid, tigecycline, and minocycline were active against >90% of VISA isolates, while >90% of VRSA isolates were susceptible to ceftaroline, daptomycin, linezolid, minocycline, rifampin, and trimethoprim/sulfamethoxazole. There were no VISA or VRSA isolates that carried PVL genes or ACME, and most strains (69.8%) were staphylococcal cassette chromosome mec type II. VRSA isolates were predominantly related to USA100 (53.8%) and none were related to USA300 or USA400.

Conclusions. A large number of available antimicrobial agents retain very good in vitro activity against VRSA and VISA isolates. The present isolates appear to be derived from healthcare-associated strains based on the absence of features associated with community-associated strains, and VRSA isolates are polyclonal by PFGE.

Vancomycin is currently the most frequently prescribed antimicrobial agent at academic medical centers in the United States. With more than 50 years of use, it is not surprising that there has emerged isolates of the most common pathogen treated with this agent, Staphylococcus aureus, with reduced in vitro susceptibility, intermediate resistance, and now complete resistance to this agent [1, 2]. Vancomycin-intermediate Staphylococcus aureus (VISA) and vancomycin-resistant Staphylococcus aureus (VRSA) strains present a therapeutic challenge to the infectious disease clinician. In addition, the molecular epidemiology of these strains is poorly understood, which limits our ability to identify patients at high risk for VISA and VRSA infections. The recent description of VISA phenotype among the community-associated clone USA300 highlights the difficulty in predicting reduced vancomycin susceptibility [3, 4]. The molecular typing and in vitro activity of approved antimicrobial agents against available isolates of VISA and a large panel of VISA have not been summarized to date. We evaluated the in vitro susceptibility of VISA and VRSA strains to commercially available antimicrobial agents that might be considered when treating these pathogens. We also evaluated molecular features including the luk-PV genes encoding Panton-Valentine leukocidin (PVL), the arcA gene of the arginine catabolic mobile element (ACME), staphylococcal cassette chromosome (SCC) mec typing, and pulsed-field gel electrophoresis (PFGE).

METHODS

Isolates
A collection of 33 VISA strains and 13 VRSA strains were selected for evaluation. All available VISA and
VRSA isolates that could be obtained through the Network on Antimicrobial Resistance in S. aureus (NARSA) Program were included. The VISA strains were cultured from blood (n = 12), wound (n = 5), bile (2), peritoneal fluid (n = 1), bone (n = 1), cerebrospinal fluid (n = 1), respiratory (n = 1), urine (1), and 9 unknown sources. The VRSA strains were cultured from wounds (n = 8), catheter site (n = 1), urine (n = 1), nephrotomy tube (n = 1), and prosthetic knee drainage (n = 2). Reference USA100 through USA1100 isolates used as controls for PFGE were also obtained from NARSA.

**Antimicrobial and In Vitro Susceptibility Testing**

In vitro susceptibility testing was performed with the following antimicrobials: ceftaroline, clindamycin, daptomycin, linezolid, minocycline, moxifloxacin, rifampin, trimethoprim/sulfamethoxazole, tigecycline, vancomycin, and telavancin. Microdilution tests using cation-adjusted Mueller-Hinton (MH) broth were used to determine the minimal inhibitory concentration (MIC) of all antimicrobial agents except telavancin. The MICs of telavancin were determined using E-test strips because commercial telavancin powder for in vitro testing was not available. For the testing of daptomycin, additional calcium was added to the MH broth to a final concentration of 50 mg/L. Microtiter plates were inoculated with 5×10^5 colony-forming units (CFU)/mL and incubated in air at 35°C for 18–24 hours. MICs were determined in accordance with Clinical and Laboratory Standard Institute (CLSI) guidelines [5] and read visually as the lowest drug concentration well with no visible bacterial growth. S. aureus ATCC 29213 and Enterococcus faecalis ATCC 29212 were used to monitor quality control for all antibiotics with each run. Quality control results were in the acceptable range for all antibiotics.

**Molecular Testing**

A multiplex polymerase chain reaction (PCR) procedure was used to determine the SCC meca typing of all isolates [6]. The PVL genes lukS-PV and lukF-PV were detected by PCR [6]. The presence of the arginine catabolic mobile element (ACME) was determined by PCR detection of the arca locus [6]. DNA used for the PCR assays was extracted from isolates using a Qiagen QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following manufacturer’s instructions.

The VRSA isolates and the USA isolates 100 through 1100 were further analyzed by PFGE using the restriction enzyme Smal. The PFGE gel patterns were compared with the development of a dendrogram using GelCompar II software (Applied Maths). Percent similarities were derived from the unweighted pair group method using arithmetic averages (UPGMA) and based on Dice coefficients. Band position tolerance was set at 1.25 and optimization was set at 0.5% [7]. Isolates were determined to belong to the same PFGE strain group if their similarity coefficient was ≥80%.

**RESULTS**

The in vitro activities of the antimicrobials are summarized in Table 1. Among the 33 VISA strains tested, only telavancin and linezolid demonstrated activity against 100% of the isolates. In addition, more than 90% of VISA isolates were susceptible to tigecycline and minocycline. Noteworthy is the fact that other agents that are commonly utilized for the treatment

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>VISA MIC Range</th>
<th>VISA % Susceptible</th>
<th>VISA MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>VISA MIC&lt;sub&gt;90&lt;/sub&gt;</th>
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</thead>
<tbody>
<tr>
<td>Linezolid</td>
<td>0.5–4</td>
<td>100</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Telavancin</td>
<td>0.25–1</td>
<td>100</td>
<td>0.5</td>
<td>0.75</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.03–1</td>
<td>97</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.03–16</td>
<td>94</td>
<td>0.12</td>
<td>4</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>0.25–2</td>
<td>85</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>0.06/1.2–4/76</td>
<td>70</td>
<td>0.25/4.8</td>
<td>&gt;4/76</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&lt;0.004–4</td>
<td>51</td>
<td>1</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.06–&gt;64</td>
<td>30</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>1–8</td>
<td>30</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.25–16</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4–8</td>
<td>0</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
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Abbreviations: MIC, minimal inhibitory concentration; MIC<sub>50</sub>, concentration needed to inhibit 50% of isolates; MIC<sub>90</sub>, concentration needed to inhibit 90% of isolates; VISA, vancomycin-intermediate Staphylococcus aureus.
of MRSA, that is, ceftaroline, trimethoprim/sulfamethoxazole, and daptomycin, had resistance rates of 15%, 30%, and 70%, respectively. The results for the 13 VRSA isolates were distinct from the VISA results (Table 2). Ceftaroline, daptomycin, linezolid, minocycline, and trimethoprim/sulfamethoxazole demonstrated activity against all isolates tested. In addition, tigecycline and rifampin were active against 92% of the VRSA strains. Telavancin demonstrated poor activity against VRSA isolates using existing breakpoints.

Among the 13 VRSA isolates, PFGE demonstrated 5 pairs of strains with ≥90% relatedness and 3 unique strains (Figure 1). Seven isolates demonstrated >80% relatedness to USA100 and 1 was >80% related to USA800. No isolates demonstrated >80% relatedness to USA300 or USA400. Among the 33 VISA isolates, 4 (12.1%) were methicillin-susceptible Staphylococcus aureus (MSSA), 21 (63.6%) were SCCmec type II, 5 (9.1%) each were types I and III, and 2 were type IVd. Among the VISA isolates, there were no MSSA, 9 (69.2%) type II, and 2 (15.4%) type IV (2 were untypable). All of the VRSA and VISA isolates were PVL and ACME negative.

DISCUSSION

The continued threat of resistant S. aureus infections is of concern to clinicians, thus the epidemiology and therapeutic options against these organisms need continued investigation. In this study, telavancin and linezolid were found to be active against all VISA strains and tigecycline and minocycline were active against more than 90% of the strains. Among the VRSA isolates, ceftaroline, daptomycin, linezolid, minocycline, trimethoprim/sulfamethoxazole, rifampin, and tigecycline were active against more than 90% of the isolates. The role of bacteriostatic agents such as minocycline and tigecycline is limited in the treatment of serious bacterial infections, as is demonstrated by the higher minimum bactericidal concentrations. This may not be the case for linezolid, which is bacteriostatic in vitro but has been reported to be bacteriocidal in serum [8].

There are several other series that report susceptibility data of available antibiotics against VISA and VRSA strains [1, 9–14]. All of these included VRSA isolates (ranging from 3 to 10) and 3 included VISA isolates. Among the studies evaluating VISA susceptibility data, 2 reported combined susceptibility data for vancomycin heteroresistant and VISA isolates [9, 10]. In the prior studies, a range of 1–7 approved anti-MRSA therapies were tested against the isolates. In the 1 study evaluating 14 VISA isolates against 5 available antibiotics, the values for MIC range and MIC90 were similar to the values reported in Table 1 [13]. Among the 6 studies evaluating VRSA isolates, only 2 had a sufficient number of isolates (7 and 10) and available antibiotics other than vancomycin tested (4 and 5) [1, 13]. Again, susceptibility results including MIC range and MIC90 were similar to those reported in Table 2. Of prior studies, there are only single studies that compared VRSA susceptibility results with those of newer agents such as telavancin [11], tigecycline [13], and ceftaroline [14]. Our study represents the largest collection of VRSA and VISA isolates tested against the widest range of available antistaphylococcal antibiotics.

The increased activity of ceftaroline and daptomycin against VRSA compared with VISA isolates likely relates to different mechanisms of resistance. Although the transfer of vanA to S. aureus isolates is the proven mechanism for development of the VRSA phenotype [1], VISA is due to increased cell wall thickness [2]. Similar susceptibility results against 7 VRSA
concerns that USA300 and USA400 will develop increasing nature and widespread distributions of these strains has raised mecPVL, ACME, and the SCC related disease [17]. These strains typically carry the genes for skin and soft tissue, pneumonia, and now healthcare-resulted in a substantial increase in MRSA infections, including predominantly due to clones USA300 and USA400 has resulted in a substantial increase in MRSA infections, including skin and soft tissue, pneumonia, and now healthcare-related disease [17]. These strains typically carry the genes for PVL, ACME, and the SCC mec type IV [18]. The virulent nature and widespread distributions of these strains has raised concerns that USA300 and USA400 will develop increasing vancomycin resistance. There have been no prior studies evaluating for the presence of PVL and ACME among VRSA or VISA isolates. Although 2 VRSA strains in this study did demonstrate SCC mec type IV, no VISA or VRSA isolates carried the genes for PVL or ACME. At present, USA300 isolates with vancomycin MIC \( \geq 2 \mu g/mL \) appear to be uncommon [3, 4]. Ongoing surveillance to evaluate larger numbers of USA300 and USA400 isolates should continue in order to detect trends in vancomycin MICs.

In summary, the present study confirms the activity of a number of approved antistaphylococcal agents against both VISA and VRSA. Confirmation of clinical activity with these agents is needed in patients with serious VISA and VRSA infections. Although infections due to VRSA do not appear to be clonal, continued surveillance for outbreaks of infection is needed. The present study is limited by the absence of clinical information to determine if there is any geographic relationship among these pairs of isolates and outbreaks of VRSA cases have not been reported to date. Future studies to evaluate VRSA should include clinical epidemiology in order to determine if cases are sporadic events or represent person-to-person spread. When clinicians encounter VISA and VRSA isolates, the results of this study may help guide empiric therapy until confirmatory susceptibility tests are performed against other agents.

isolates with 5 of the antimicrobial agents used in this study have been described previously [1]. The high level of telavancin resistance among VRSA isolates is not surprising because telavancin has a dual mechanism of action that includes inhibition of cell wall synthesis [15]. As reported previously, telavancin does retain excellent activity against MRSA isolates with vancomycin MIC \( \geq 2 \) that are not VRSA phenotypes [16].

Previous typing of VRSA isolates using PFGE was limited to 7 isolates [1], whereas the present study evaluated all 13 available VRSA isolates using USA100 through USA1100 for comparison. In the prior study that evaluated molecular epidemiology, 5 of 7 isolates were related to USA100 and were SCC mec type II and I was related to USA800 and was SCC mec type IV (1 was untypable). This is similar to our data on a larger number of isolates.

The global spread of community-associated MRSA infections predominantly due to clones USA300 and USA400 has resulted in a substantial increase in MRSA infections, including skin and soft tissue, pneumonia, and now healthcare-related disease [17]. These strains typically carry the genes for PVL, ACME, and the SCC mec type IV [18]. The virulent nature and widespread distributions of these strains has raised concerns that USA300 and USA400 will develop increasing strains of all vancomycin-resistant Staphylococcus aureus isolates and USA100 through USA1100 isolates. Abbreviation: VRSA, vancomycin-resistant Staphylococcus aureus.

**Figure 1.** Dendrogram comparing pulsed-field gel electrophoresis patterns of all vancomycin-resistant *Staphylococcus aureus* isolates and USA100 through USA1100 isolates. Abbreviation: VRSA, vancomycin-resistant *Staphylococcus aureus*.

**Notes**

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**Potential conflicts of interest.** All authors: No reported conflicts.

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**References**


6. Saravolatz LD, Pawlak J, Johnson LB. In vitro activity of oritavancin against community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), vancomycin-intermediate *S. aureus* (VISA), and USA300 and USA400 isolates should continue in order to detect trends in vancomycin MICs.