Methicillin-Susceptible, Vancomycin-Resistant

*Staphylococcus aureus*, Brazil

Diana Panesso, Paul J. Planet, Lorena Diaz, Jean-Emmanuel Hugonnet, Truc T. Tran, Apurva Narechania, Jose M. Munita, Sandra Rincon, Lina P. Carvajal, Jinnethe Reyes, Alejandra Londoño, Hannah Smith, Robert Sebra, Gintaras Deikus, George M. Weinstock, Barbara E. Murray, Flavia Rossi, Michel Arthur, Cesar A. Arias

We report characterization of a methicillin-susceptible, vancomycin-resistant bloodstream isolate of *Staphylococcus aureus* recovered from a patient in Brazil. Emergence of vancomycin resistance in methicillin-susceptible *S. aureus* would indicate that this resistance trait might be poised to disseminate more rapidly among *S. aureus* and represents a major public health threat.

Acquisition of high-level vancomycin resistance by *Staphylococcus aureus* represents a major public health risk because this antimicrobial drug continues to be the first-line and most inexpensive therapy to treat methicillin-resistant *S. aureus* (MRSA) despite concerns about its clinical efficacy. Recently, we described vancomycin-resistant MRSA (VR-MRSA) recovered from the bloodstream of a patient in Brazil (1). VR-MRSA belongs to sequence type (ST) 8 and is phylogenetically related to the community-associated (CA) MRSA USA300 genetic lineage that has rapidly disseminated in the United States and the northern region of South America (USA300-Latin American variant [USA300-LV]) (1,2). The vanA gene cluster in VR-MRSA was carried by a transferable staphylococcal plasmid (pBRZ01). We characterized a clinical isolate of vancomycin-resistant, methicillin-susceptible *S. aureus* (VR-MSSA) and document the in vivo transfer of the vanA gene cluster to 2 unrelated *S. aureus* strains causing bacteremia within the same patient.

The Study

On August 28, 2012, a blood culture from a patient in Brazil was reported positive for 2 isolates of MSSA while the patient was receiving daptomycin therapy (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/10/14-1914-Techapp1.pdf). One MSSA isolate was susceptible to all antimicrobial drugs tested (VS-MSSA). The second isolate (VR-MSSA) had a vancomycin MIC of 256 µg/mL and was also resistant to gentamicin (Table 1). Both isolates were susceptible to daptomycin (MIC 0.5 µg/mL). Thirteen days earlier, 2 MRSA isolates, 1 of which was resistant to vancomycin (VR-MRSA), were recovered from the blood of the same patient (online Technical Appendix) (1). The daptomycin MICs for both MRSA strains were also 0.5 µg/mL.

Bacterial strains used in this study (Table 1) were grown in brain–heart infusion broth and agar. Plasmid pBRZ01 was transferred by using filter mating (3) and VR-MRSA and VR-MRSA as donors and VS-MSSA, VS-MRSA, and RN4220RF as recipients (Table 1). Transconjugants were selected on brain heart infusion medium containing vancomycin (32 µg/mL) and fusidic acid (25 µg/mL). Colonies from each mating experiment were subjected to digestion with Smal and pulsed-field gel electrophoresis to investigate genetic relatedness (1). Plasmids carrying the vanA gene cluster were detected by using S1 nuclease digestion followed by hybridization with a vanA probe (4).

Whole-genome sequencing of VR-MSSA, VS-MSSA, and 2 representatives of the Chilean/Cordobes clone (M1, M91) was performed by using MiSeq PacBio RS II (Illumina, San Diego, CA, USA) to close the VR-MSSA genome (5) (online Technical Appendix). Phylogenetic analysis was performed by using the maximum-likelihood framework within RAxML v7.4.2 (6). For cell wall analysis, extraction and separation of peptidoglycan precursors was performed as described (7).

The PFGE patterns of both isolates (VR-MSSA and VS-MSSA) were indistinguishable, and in vitro growth rates were similar (Figure 1, panel A). S1 nuclease analyses indicated that VR-MSSA harbored a plasmid of ≈55

DOI: http://dx.doi.org/10.3201/eid2110.141914
kb, which yielded a positive result when hybridized with a vanA probe (Figure 1, panels B, C) and was similar in size to the previously described vanA-containing plasmid pBRZ01 identified in the same patient (1). pBRZ01 of VR-MSSA was readily transferred to S. aureus RN4220-RF (efficiency = 3 × 10^{-5}/donor). In vitro conjugative transfer of pBRZ01 between MRSA and MSSA strains recovered from the patient’s bloodstream was also readily achieved with efficiencies ranging from 4.3 × 10^{-7}/donor to 2.5 × 10^{-6}/donor. Acquisition of the pBRZ01 by corresponding strains resulted in resistance to vancomycin and gentamicin (Table 1).

**Table 1. Staphylococcus aureus** strains used in analysis of methicillin and vancomycin resistance, Brazil*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Strain characteristics</th>
<th>MIC, µg/mL</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS-MRSA</td>
<td>Isolated from the bloodstream of a patient in Brazil</td>
<td>0.5</td>
<td>(1)</td>
</tr>
<tr>
<td>VR-MRSA</td>
<td>Isolated from the blood of the same patient above and carrying vanA-containing pBRZ01</td>
<td>&gt;256</td>
<td>32</td>
</tr>
<tr>
<td>VS-MSSA</td>
<td>Isolated from the blood of the same patient 13 d after isolation of VR-MRSA</td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>VR-MSSA RN4220-RF</td>
<td>Isolated from the same blood culture as VS-MSSA</td>
<td>256</td>
<td>48</td>
</tr>
<tr>
<td>Transconjugant 1†</td>
<td>Transconjugant obtained from a mating experiment using VR-MSSA as donor and VS-MRSA as recipient</td>
<td>&gt;256</td>
<td>48</td>
</tr>
<tr>
<td>Transconjugant 2†</td>
<td>Transconjugant obtained from a mating experiment using VR-MRSA as donor and VS-MSSA as recipient</td>
<td>&gt;256</td>
<td>64</td>
</tr>
<tr>
<td>Transconjugant 3†</td>
<td>Transconjugant obtained from a mating experiment using VR-MSSA as donor and RN4220-RF as recipient</td>
<td>&gt;256</td>
<td>64</td>
</tr>
</tbody>
</table>

*VS-MRSA, vancomycin-resistant, methicillin-resistant S. aureus; VS-MSSA, vancomycin-susceptible, methicillin-resistant S. aureus; VR-MSSA, vancomycin-resistant, methicillin-susceptible S. aureus.
†A fusidic acid–resistant derivative was generated for mating experiments. All mating experiments were performed on brain heart infusion agar in the presence of vancomycin (32 µg/mL) and fusidic acid (25 µg/mL) to select for transconjugants.

Figure 1. Molecular typing of Staphylococcus aureus strains, Brazil. A) Smal digestion of total DNA, followed by pulsed-field gel electrophoresis. Lane M, lambda ladder (molecular masses are indicated in kilobases on the left); lane 1, vancomycin-susceptible, methicillin-resistant S. aureus (VS-MRSA) isolated from the blood of a Brazilian patient (1); lane 2, vancomycin-resistant MRSA (VR-MRSA) isolated from the same patient and blood culture (1); lane 3, transconjugant 1 obtained from a mating experiment using vancomycin-resistant MSSA (VR-MSSA) as donor and VS-MRSA as recipient; lane 4, vancomycin-susceptible MSSA (VS-MSSA) isolated from the blood of the same patient 13 days after isolation of VR-MRSA; lane 5, VR-MSSA isolated at the same time as VS-MRSA; lane 6, transconjugant 2 obtained from a mating experiment using VR-MRSA as donor and VS-MSSA as recipient; lane 7, S. aureus RN4220 RF, lane 8, transconjugant 3 obtained using VR-MSSA as donor and RN4220 RF as recipient. B) S1 digestion of total DNA using the same strains shown in panel A. C) Hybridization with vanA probe using the same strains shown in panel A. Arrow indicates a positive signal for the vanA gene.
(isolated days before from the same patient) and emphasized the relationship of this strain to other vancomycin-resistant *S. aureus* and MRSA isolates with intermediate susceptibility to vancomycin (VISA).

We analyzed the pool of cytoplasmic peptidoglycan precursors of VR-MSSA grown in the absence or presence of 50 μg/mL of vancomycin for induction of the *vanA* cluster (Table 2). Tandem mass spectrometry analysis identified 3 nucleotide precursors ending in d-alanyl-d-alanine (UDP-MurNAc-pentapeptide), d-alanyl-d-lactate (UDP-MurNAc-pentadepsipeptide), and d-Ala (UDP-MurNAc-tetrapeptide). Upon induction with vancomycin, UDP-MurNAc-pentapeptide was not detected, and UDP-MurNAc-pentadepsipeptide accounted for most of the precursors (Table 2). These results indicate that the *van*-encoded enzymes required for incorporation of d-Lac into the precursors were fully functional in VR-MSSA. Our results also show that the *vanA* cluster was inducible by vancomycin in the *S. aureus* host because only a small proportion of the precursors (4%) ended in d-Lac in the absence of the drug.

Analyses of cell wall muropeptides from VR-MSSA showed 2 modifications of the L-Ala<sub>1</sub>-γ-d-Glu<sub>2</sub>-L-Lys<sub>3</sub>-d-Ala<sub>4</sub>-d-Ala<sub>5</sub> stem peptide that are highly conserved in *S. aureus* strains, namely the amidation of the α-carboxyl of d-Glu to form d-iGln<sub>2</sub> and the addition of a pentaglycine side chain on the ε-amino group of L-Lys<sub>3</sub> by the Fem amino-acyltransferases (9). Induction of the *vanA* gene cluster

---

**Figure 2.** Phylogenetic analyses of *Staphylococcus aureus* strains, Brazil. Whole-genome phylogenetic tree (dataset = 325,732 single-nucleotide polymorphisms, gamma-based log likelihood = −1906607.06950) of the *S. aureus* species showing position of vancomycin-resistant, methicillin-susceptible *S. aureus* (VR-MSSA) and vancomycin-susceptible MSSA (VS-MSSA) isolates sequenced for this study. Vancomycin-resistant *S. aureus* (VRSA) strains are shown in red. Numbers on branches are bootstrap values based on 1,000 resampling iterations. All branches without numbers had bootstrap values of 100%. Branch lengths are proportional to number of nucleotide substitutions per site (scale bars). Inset labeled CC5 is expanded to emphasize the polyphyly of VRSA strains. *Genomes sequenced for this study. M1 and M91 are members of the Chilean/Cordobes clone that is widespread in Latin America (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/10/14-1914-Techapp1.pdf). CC, clonal complex.
Table 2. Relative abundance of peptidoglycan precursors in *Staphylococcus aureus* strains, Brazil

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Monoisotopic mass</th>
<th>Abundance (%) in corresponding strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Calculated</td>
</tr>
<tr>
<td>UDP-MurNAc-tetrapeptide</td>
<td>1,078.35</td>
<td>1,078.35</td>
</tr>
<tr>
<td>UDP-MurNAc-pentapeptide</td>
<td>1,149.37</td>
<td>1,149.39</td>
</tr>
<tr>
<td>UDP-MurNAc-pentadepsipeptide</td>
<td>1,150.37</td>
<td>1,150.37</td>
</tr>
</tbody>
</table>

*Bacteria were grown on brain heart infusion (BHI) broth (not induced) or BHI supplemented with vancomycin (50 μg/mL). VS-MSSA, vancomycin-susceptible, methicillin-susceptible *S. aureus*; VR-MSSA, vancomycin-resistant, methicillin-susceptible *S. aureus*; ND, not detected.

led to 2 major modifications. First, stem peptides ended in d-Ala5, indicating that the peptidyl- d-Ala5- d-Ala5 target of vancomycin, and d-Ala5-d-Lac5 termini, were fully eliminated. Second, the pentaglycine side chain was frequently missing (online Technical Appendix), indicating that replacement of d-Ala by d-Lac at the extremity of peptidoglycan precursors might have impaired the ability of Fem transferases to add Gly on L-Lys3.

Conclusions

In this study, we demonstrated that the *vanA*-containing pBRZ01 plasmid previously described in MRSA was acquired by an invasive MSSA isolate within the same patient. Our findings also suggest that a *vanA*-containing plasmid (pBRZ01) was horizontally acquired at least twice during a short period by distinct *S. aureus* lineages within the same host (MRSA belonging to ST8 and an ST5 MSSA). VR-MSSA belongs to the ST5 lineage of CC5, a major hospital-associated lineage (10). The prevalent hospital-associated lineages circulating in Brazil are ST5 (New York/Japan and Pediatric clones), ST239 (Brazilian clone) and ST1 (USA400 clone) (11), and recent epidemiologic data showed replacement of the endemic Brazilian (ST239) clone by ST5 strains (11–13). Moreover, VR-MSSA is related to ST5 vancomycin-resistant *S. aureus* strains recovered in the United States (8) and to VISA isolates, including Mu50 and the hetero-VISA strain Mu3, initially recovered in Japan (14). It remains unclear why CC5 strains appear more likely to exhibit vancomycin resistance.

Our biochemical analysis indicates that the *vanA* gene cluster is fully functional in VR-MSSA, which leads to vancomycin-inducible production of d-Lac ending precursors and elimination of d-Ala- d-Ala containing peptidoglycan, as found in the enterococci (15). Our results also revealed a defect in side chain synthesis, although this did not prevent the synthesis of a functional and highly cross-linked peptidoglycan in VR-MSSA.

In summary, we report the in vivo acquisition of high-level vancomycin resistance in a bloodstream MSSA isolate. Of note, *vanA*-containing pBRZ01 was maintained even after the selective pressure of vancomycin had been removed, raising serious concerns about the possibility of further spread of resistance to this agent. However, no other MSSA strains containing this plasmid have been isolated so far in Brazil.

C.A.A. is supported by National Institutes of Health–National Institute of Allergy and Infectious Diseases (NIH-NIAID) grant R01 AI093749, B.E.M. is supported by NIH-NIAID grant R01 AI047923, and P.J.P. is supported by NIH-NIAID grant K08AI101005.

Dr. Panesso is a postdoctoral researcher at the Laboratory for Antimicrobial Research, University of Texas Medical School at Houston and associate professor of research at the Molecular Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Bogota, Colombia. Her research interests include the molecular aspects of antimicrobial resistance, with emphasis on gram-positive bacteria.

References

7. Bouhss A, Josseuma N, Severin A, Tabei K, Hugonnet JE, Bosque, Bogota, Colombia. Her research interests include the Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Bogota, Colombia. Her research interests include the molecular aspects of antimicrobial resistance, with emphasis on gram-positive bacteria.


Address for correspondence: Cesar A. Arias, University of Texas Medical School at Houston, 6431 Fannin St, MSB 2.112. Houston, TX 77030, USA; email: cesar.arias@uth.tmc.edu

July 2015: Malaria

Lack of Transmission among Close Contacts of Patient with Case of Middle East Respiratory Syndrome Imported into the United States, 2014

Monitoring of Ebola Virus Makona Evolution through Establishment of Advanced Genomic Capability in Liberia

Parechovirus Genotype 3 Outbreak among Infants, New South Wales, Australia, 2013–2014


Assessment of Arbovirus Surveillance 13 Years after Introduction of West Nile Virus, United States

Results from the National Legionella Outbreak Detection Program, the Netherlands, 2002–2012

Seroprevalence for Hepatitis E and Other Viral Hepatitides in Diverse Populations, Malawi

Swine Influenza A(H3N2) Virus Infection in an Immunocompromised Man, Italy, 2014

Severe Pediatric Adenovirus 7 Disease in Singapore Linked to Recent Outbreaks across Asia

Hemagglutinin Receptor Binding of a Human Isolate of Influenza A(H10N8) Virus

Schmallenberg Virus Reoccurrence, Germany 2014

Detection of Circovirus in Foxes with Meningoencephalitis, United Kindom, 2009–2013

Readability of Ebola Information on Websites of Public Health Agencies, United States, United Kingdom, Canada, Australia, and Europe

Find these and more articles at http://wwwnc.cdc.gov/eid/articles/issue/21/7/table-of-contents