Healthcare-associated Endocarditis Caused by *Staphylococcus aureus* with Reduced Susceptibility to Vancomycin

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**Key words:** endocarditis, *Staphylococcus aureus*, vancomycin, linezolid

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Infective endocarditis (IE) caused by methicillin-resistant *Staphylococcus aureus* with reduced vancomycin susceptibility (SARV) has been reported worldwide. We report successful treatment of a pediatric patient with SARV IE and strain characterization. The MIC of vancomycin rose from 1.5 to 2µg/mL and the SARV was confirmed by population analysis.
A 5-year-old girl with complicated congenital heart disease was admitted to our hospital for re-do coarctation of aorta repair. She attended our outpatient department every 3 months for more than one year before her admission this time. Two weeks after the operation, she developed sternal wound methicillin-resistant *Staphylococcus aureus* (MRSA) infection (isolate A1160). Transthoracic echocardiography (TTE) revealed no vegetation initially. She received 6 weeks of vancomycin therapy (10 mg/kg/dose, q6h) and the wound culture was sterile after that treatment. One week after completing vancomycin therapy, the patient suffered from high fever. Blood culture revealed MRSA, and vancomycin and rifampin combination therapy was initiated (day 1). TTE performed one week later revealed a 0.529 cm² vegetation on pulmonary valve. Blood cultures performed on days 11 to 13 after starting the vancomycin and rifampin combination therapy still yielded MRSA (isolate A1230) and intermittent high fever was still noted. The vancomycin dose was changed to 15 mg/kg/dose on day 15 due to inadequate level (trough level, 9.23 µg/mL). Surveillance blood cultures performed on days 16 and 21 were negative. However, fever and MRSA bacteremia recurred on day 24 (isolate A1234). The MRSA isolates A1230 and A1234 demonstrated antimicrobial susceptibility profiles similar to the patient’s first isolate A1160 (Table 1), except that the minimal inhibitory concentration (MIC) of vancomycin was 1.5 µg/mL in A1160 and 2 µg/mL in A1230 and A1234, and rifampin MIC increased from < 0.016 µg/mL in A1160 and A1230 to >256 µg/mL in A1234 determined by the Etest method (AB Biodisk). The organisms were also resistant to clindamycin, clarithromycin, gentamicin, and ciprofloxacin, but were susceptible to fucidic acid, teicoplanin and linezolid (MIC 1~1.5 µg/mL). TTE was performed again and still revealed vegetation at the same site.
We then switched the therapeutic regimen to linezolid (10 mg/kg, iv, q8h) plus fucidic acid for 42 days followed by oral formulation for an additional 14 days. The patient became afebrile and blood culture was sterile within 48 hours after starting linezolid and fucidic acid combination therapy. Subsequent surveillance blood cultures performed during this combination therapy all yielded no growth. The patient tolerated the intravenous infusion of linezolid without adverse effect. Weekly monitored liver, renal and hematological parameters were normal during the treatment course. TTE obtained 2 weeks after the end of therapy revealed complete resolution of the vegetation.

Isolates A1160, A1230, and A1234 were subjected to further genotypic testing. Virulence and toxin genes, and the macrolide-lincosamide-streptogramin (MLS) resistance genes (ermA, ermB, ermC), and staphylococcal cassette chromosome mec (SCCmec) typing were determined by PCR amplification. All three isolates possessed fnbA, fnbB, sea and seb genes. These isolates were positive for ermA but were negative for lukFS-PV. All three isolates carried SCCmec type III and had indistinguishable pulsed field gel electrophoresis (PFGE) patterns. These results indicated that the same strain of MRSA was responsible for the initial sternal infection and subsequent bacteremia and endocarditis.

Because of the poor clinical response to vancomycin treatment, we performed population analysis profile (PAP) study on the 3 MRSA isolates to test for reduced susceptibility to vancomycin (9,10). The PAP area under the curve (AUC) of the test strains was compared to the AUC of Mu3, a heterogenous vancomycin-intermediate S. aureus (hVISA) (ATCC 700698) strain. All three MRSA strains fit the criteria of hVISA, which was defined by vancomycin MIC of ≤2 µg/mL and the AUC of the test strain to Mu3 was >0.9 (10,21). The first two isolates A1160 and A1230 had AUC ratio of >0.9,
while the last isolate A1234 had AUC ratio of >1. The increased AUC ratio of the last 
strain indicated further emergence of a subpopulation of isolates with reduced
susceptibility to vancomycin.

MRSA endocarditis is a serious, potentially life-threatening clinical event. Currently, 
vancomycin is the leading treatment of choice. The emergence of isolates with reduced 
susceptibility to vancomycin (SARV), including hVISA and VISA, among patients with 
endocarditis has become problematic around the world (1,6,11). The possible reason for 
the emergence of SARV and ideal therapy for patients with endocarditis due to SARV are 
still unknown. We report the successful treatment with a combination of linezolid and 
fucidic acid for a patient with hospital-associated SARV endocarditis. Our
characterization of the causative strain in clinical sequence also provides an association
between failure of vancomycin therapy and the emergence of SARV.

Similar to other patients with hVISA, VISA or SARV infections (3,12,20), our 
patient was treated with an extended course of vancomycin in the context of nosocomial 
MRSA infection. Although a thickened cell wall and the loss of accessory gene regulator 
function are notable common features (7,8,15,16,19), the precise mechanisms of hVISA 
resistance to vancomycin have not been elucidated. Previous studies have shown that low 
vancomycin trough levels (<10 μg/mL), particularly in the early stages of therapy, could 
result in induction of pre-existing low-level vancomycin resistance or could select for 
new vancomycin-resistant strains (8,11). Subinhibitory concentration of vancomycin 
could be an important factor leading to the selection of SARV strains. Besides, even the 
isolates were fully susceptible to vancomycin (MIC <2 μg/mL), MRSA strains with
vancomycin MIC of <0.5 µg/mL responded much more readily than did MRSA strains with MIC of 1-2 µg/mL (17). Subtle changes in increasing vancomycin MIC well within the susceptible range and differences in susceptibility to killing may contribute to clinical failure (16). In the present case, we provide a clear clinical sequence. We suggest that a healthcare-associated vancomycin-susceptible S. aureus strain with higher vancomycin MIC and reduced susceptibility subpopulation was primarily responsible for the infection initially but ineffective vancomycin therapy selected for the SARVs and resulted in glycopeptide treatment failure.

Of noteworthy is that resistance to rifampin also developed during vancomycin and rifampin combination therapy in our case. In a previous study, in vitro antagonism had been shown when rifampin was added to vancomycin (18). In a prospective, randomized trial, Levine et al. demonstrated that the addition of rifampin to vancomycin was counterproductive in patients with MRSA endocarditis. Fever was sustained and bacteremia was prolonged (13). Howden et al. also reported resistance to rifampin developing during vancomycin and rifampin combination therapy in three cases with bacteremia (11). Emergence of resistance and prolonged bacteremia appeared to be risk factors contributed to our patient’s failure. Thus, in attempts to improve the bactericidal capacity in patients with endocarditis, the regimens of combination therapy require further evaluation.

Our patient was treated for eight weeks with linezolid and fucidic acid combination therapy with apparent resolution of the vegetations as demonstrated by echocardiography.

In a previous report, the addition of either rifampin or TMP-SMX to the linezolid regimen was considered as alternatives to treat patients with endocarditis (20). For this patient, we used linezolid and fucidic acid because the isolates were resistant to many
antibiotics. Moreover, the availability of switching to an oral formulation allowed an early discharge.

The role of linezolid in the treatment of endocarditis caused by multidrug-resistant gram-positive pathogens is unresolved. Although animal and clinical studies showed therapeutic efficacy of linezolid in endocarditis (2,14), clinical treatment failures have also been reported recently (4). Therefore, linezolid should be used prudently in selected patients, and testing for bactericidal capacity of linezolid with other antistaphylococcal agents combinations is suggested.

Daptomycin has been approved for use in patients with MRSA bacteremia, including that associated with right-sided endocarditis (5). However, the usual dose for pediatric patients is unknown and the efficacy for SARV should have further evaluation.

The emergence of SARV is becoming a challenge to the clinicians. Monitoring of therapeutic level and adjusting the dosage of vancomycin in treating complicated cases is warranted. An alternative regimen for SARV is needed for inoperable patients, and linezolid could be considered a therapeutic option. With the increasing recognition of SARV, further characterizations will help predict which isolates are most likely to respond.
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REFERENCES


TABLE 1. Antibiograms of *Staphylococcus aureus* isolates obtained from a pediatric patient with endocarditis in time sequence

<table>
<thead>
<tr>
<th>Isolate</th>
<th>A1160</th>
<th>A1230</th>
<th>A1234</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source</td>
<td>Sternal wound</td>
<td>Blood</td>
<td>Blood</td>
</tr>
<tr>
<td>Time sequence</td>
<td>Prior to a 6-week vancomycin therapy course</td>
<td>13 days after vancomycin and rifampin therapy</td>
<td>24 days after vancomycin and rifampin therapy</td>
</tr>
</tbody>
</table>

**MIC*<sup>a</sup>, µg/mL**

<table>
<thead>
<tr>
<th>Drug</th>
<th>A1160</th>
<th>A1230</th>
<th>A1234</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1.5</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Rifampin</td>
<td>&lt;0.016</td>
<td>&lt;0.016</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Fucidic acid</td>
<td>0.094</td>
<td>0.094</td>
<td>0.125</td>
</tr>
</tbody>
</table>

*<sup>a</sup> MIC, minimal inhibitory concentration determined by Etest method. TMP-SMX, trimethoprim-sulfamethoxazole.
Figure Legends:

FIG. 1. Population analysis profiles of three methicillin-resistant *Staphylococcus aureus* isolates obtained in time sequence from a pediatric patient with endocarditis. Colonies were counted after 48h of growth at 35°C. CFU, colony-forming units. ATCC 25923, vancomycin-susceptible *S. aureus*. A1160, prior to 6-week vancomycin therapy course. A1230, 13 days after vancomycin and rifampin therapy. A1234, 24 days after vancomycin and rifampin therapy.