Clinical Infectious Diseases

BRIEF REPORT

Emergence of Dalbavancin, Vancomycin, and Daptomycin Nonsusceptible *Staphylococcus aureus* in a Patient Treated With Dalbavancin: Case Report and Isolate Characterization

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A patient with end-stage renal disease received 2 doses of dalbavancin for methicillin-resistant *Staphylococcus aureus* (MRSA) arteriovenous fistula infection and presented 5 weeks later with infective endocarditis secondary to vancomycin, daptomycin, and dalbavancin nonsusceptible MRSA. Resistance was associated with *walK* and *scrA* mutations, reduced long-chain lipid content, and reduced membrane fluidity.

Keywords. dalbavancin resistance; walK mutation; lipoglycopeptide cross-resistance; dalbavancin relapse.

We have demonstrated that the lipoglycopeptide dalbavancin readily selects for cross-resistance to the glycopeptide vancomycin and the lipopeptide daptomycin in vitro using serial passage techniques and clinically relevant pharmacokinetic exposures simulated using in vitro models [1–4]. These strains usually exhibit the seesaw effect with beta-lactams and acquire mutations in genes related to the *walKR* operon, including *atl*, which is regulated by WalKR, and *stp1*, which regulates WalR phosphorylation [2]. However, there are few reported instances of dalbavancin nonsusceptibility reported in the clinical literature [4–7]. Among these reports, only 2 have utilized whole-genome sequencing, and many instances are confounded by the use of dalbavancin in the setting of multiple drug failures. Consequently, the clinical significance of dalbavancin-selected cross-resistance to vancomycin and daptomycin remains unclear. We report a new case in which a patient with end-stage renal disease received 2 doses of dalbavancin for a methicillin-resistant *Staphylococcus aureus* (MRSA)–infected arteriovenous fistula (AVF) and presented 5 weeks later with aortic valve endocarditis from an isogenic strain that was nonsusceptible to vancomycin, daptomycin, and dalbavancin.

METHODS

Patient Characteristics and Clinical Course

The patient's clinical course is summarized in Figure 1. A 63-year-old man with end-stage renal disease from autosomal dominant polycystic kidney disease, on home hemodialysis (HD) 5 times/week, was admitted on 15 February 2021 to Dayton Veteran's Affairs Medical Center with shortness of breath and cough, right-sided chest pain, and fever. Chest X-ray disclosed multifocal pneumonia. Blood cultures were taken, and he was empirically treated with ceftriaxone (2 g intravenously [IV] every 24 hours [q24h]) and azithromycin (500 mg IV q24h). On 16 February, blood cultures yielded MRSA (isolate VAHP-884) and treatment switched to renally dosed vancomycin. Computed tomography (CT) angiography of the chest and the left arm revealed several aneurysms or pseudoaneurysms of the left-upper-extremity brachiocephalic fistula venous outflow, multiple pulmonary nodules, and peripheral wedge-shaped opacities concerning for septic emboli. Transesophageal echocardiography was negative for endocarditis. Positron emission tomography (PET)/CT scan revealed avid fluorodeoxyglucose (FDG) uptake into pulmonary nodules, likely representing septic emboli, and mild FDG uptake in the left upper extremity. Repeat blood cultures on 18 February again grew MRSA, but repeat blood cultures on days 19, 21, and 23 February showed no growth. The patient underwent resection of the infected pseudoaneurysm portion of left-arm AVF with interposition bovine graft on 22 February. On 26 February, vancomycin was discontinued, dalbavancin was given, and the patient was discharged home. We elected to treat him with dalbavancin due to his reluctance to have a home IV agency for IV vancomycin and varied duration and number of HD sessions per week. He received a dose of dalbavancin 1500 mg on 26 February and 1000 mg on 15 March.

On 14 April, 33 days after the second dose of dalbavancin, the patient was readmitted to the hospital for shortness of breath and chills and he was empirically given vancomycin and meropenem. Blood cultures from admission grew MRSA (VAHP-2049), later identified as nonsusceptible to vancomycin, daptomycin, and dalbavancin. Transthoracic echocardiogram was negative for endocarditis, and PET/CT scan on

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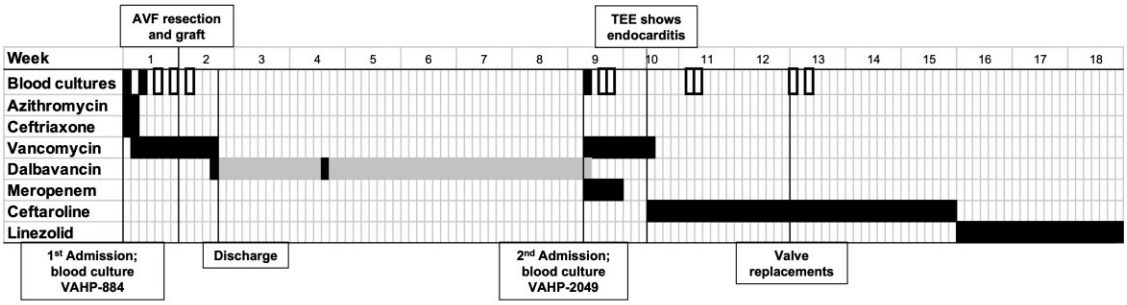

Drug	VAHP-884	VAHP-2049
Vancomycin	1 mg/L (S)	4 mg/L (NS)
Vancomycin AUC ratio	0.6 (VSSA)	0.95 (hVISA/VISA)
Daptomycin	0.25 mg/L (S)	2 mg/L (NS)
Dalbavancin	0.003 mg/L (S)	0.5 mg/L (NS)
Nafcillin (PBP-NS)	4 mg/L (R)	1 mg/L (S*)
Meropenem (PBP1)	0.5 mg/L	0.25 mg/L
Ceftriaxone (PBP2)	16 mg/L	8 mg/L
Cephalexin (PBP3)	8 mg/L	8 mg/L
Cefoxitin (PBP4)	16 mg/L (R)	8 mg/L (R)
Ceftaroline (PBP2a)	0.5 mg/L (S)	0.25 mg/L (S)
Gene (NA; AA)	----	***walK*** (C1700T; Ala567Val)
	----	***scrA*** (C569T; Ala190Val)

Figure 1. *Top.* Summary of clinical course over 18 weeks from initial presentation with shortness of breath and fever. Positive blood cultures (black filled), negative blood cultures (black outlined). The duration of each antibiotic is indicated in black, dalbavancin doses are indicated in black, and duration is highlighted in gray. Vertical lines are labeled with important events. *Bottom.* Susceptibility of parent strain VAHP-884 and post–dalbavancin exposure isolate VAHP-2049 measured by broth microdilution and vancomycin population analysis profile (AUC ratio). Minimum inhibitory concentrations are listed in mg/L, interpretation is based on established breakpoints from the Clinical Laboratory Standards Institute. *nafcillin MIC <4 mg/L is considered susceptible in methicillin-susceptible strains that do not harbor the *mecA* gene. No interpretive criteria exist for meropenem, ceftriaxone, or cephalexin against Staphylococci. WalK Ala567Val affects the histidine kinase domain close to where WalR is presumed to interact. Abbreviations: AA, amino acid change; AUC, area under the curve; AVF, arteriovenous fistula; hVISA, heterogeneous–vancomycin-intermediate *Staphylococcus aureus*; MIC, minimum inhibitory concentration; NA, nucleic acid change; NS, nonsusceptible; PBP, penicillin binding protein; R, resistant; S, susceptible; TEE, transesophageal echocardiogram; VISA, vancomycin-intermediate *Staphylococcus aureus*.

20 April disclosed no FDG avid lesions and pulmonary nodules were mostly resolved. Repeat blood cultures on 15 April and 17 April showed no growth. Transesophageal echocardiogram on 22 April revealed 2-cm aortic valve vegetation with moderate-to-severe aortic regurgitation, moderate mitral regurgitation, moderate-to-severe tricuspid regurgitation, and prolapse of the noncoronary cusp with preserved left ventricular systolic function.

The patient was transferred to an outside hospital for consideration of valve surgery and antibiotics were changed to ceftaroline-fosamil (200 mg IV every 12 hours [q12h]). On 5 October, the patient underwent median sternotomy, bioprosthetic aortic valve replacement, bioprosthetic mitral valve replacement, tricuspid valve repair using annuloplasty ring, and replacement of the ascending aorta using a woven aortic surgical graft. Histopathology of the valves revealed evidence of calcification and fibrosis but no signs of vegetation, inflammatory infiltrates, or microorganisms; however, the valves were not cultured. Postoperatively, he completed 3 weeks of ceftaroline-fosamil 200 mg IV q12h followed by 3 weeks of linezolid 600 mg orally q12h with no further positive cultures after 1 year of follow-up.

Susceptibility Testing

Susceptibilities to dalbavancin, daptomycin, vancomycin, nafcillin, meropenem, ceftriaxone, cephalexin, cefoxitin, and ceftaroline were determined in duplicate by broth microdilution per Clinical Laboratory Standards Institute guidelines. Vancomycin susceptibility by the modified population analysis profile method was performed as previously described to determine if the initial isolate was positive for the heterogeneous–vancomycin-intermediate *Staphylococcus aureus* (hVISA) phenotype [8].

Whole-Genome Sequencing

Whole-genome sequencing was performed on the patient's blood isolates using the MiSeq platform, sequence reads were mapped to Genbank accession CP043392.1, and variant calling performed as previously described [9]. Sequence features were annotated using SnpEFF [10]. Sequence data are available from the NCBI Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra; PRJNA808066).

Dalbavancin Plasma Concentration (see Supplementary Methods)

One plasma sample drawn when VAHP-2049 was isolated was available for dalbavancin quantification. The sample and calibration curve were processed as described previously but using daptomycin as the internal standard. The free fraction was estimated based on 99% protein binding reported typically reported for dalbavancin [11].

Membrane Analysis (see Supplementary Methods)

We performed comprehensive lipidomic analysis of the strain pair as described previously to evaluate the changes in cell envelope physiology and correlate membrane composition with changes in antimicrobial susceptibility and observed mutations [1, 3]. Briefly, VAHP-884 (wild-type) and VAHP-2049 (dalbavancin-resistant) were grown overnight in tryptic soy broth at 37°C with shaking, harvested by centrifugation, washed in sterile PBS, and dried by SpeedVac (Thermo Scientific). Lipids were extracted by the Bligh and Dyer method, and analyzed by hydrophilic interaction liquid chromatography coupled with ion mobility–mass spectrometry. Data were normalized to all compounds and reported as relative quantities of all lipid species including free fatty acids (FFAs), diglucosyl-diacylglycerol (DGDG), phosphatidylglycerol (PG), plasmalogen-phosphatidylglycerols (PGps), cardiolipins (CLs), and lysyl-phosphatidylglycerol (LysylPG), with fatty acyl compositions ranging from 25:0 to 38:0 (total carbons:total degree unsaturation). Membrane fluidity was measured in octuplicate by polarizing spectrofluorometry.

RESULTS

Susceptibility Testing, Whole-Genome Sequencing, and Dalbavancin Concentration

Susceptibility changes and mutations differentiating VAHP-844 and VAHP-2049 are summarized in Figure 1. There were 2 single nucleotide mutations that arose in VAHP-2049, one in *walK* and one in *scrA*. VAHP-844 was fully susceptible to vancomycin, daptomycin, and dalbavancin and was a non-hVISA by vancomycin population analysis (area under the curve [AUC] ratio <0.9). VAHP-2049 was vancomycin-, daptomycin-, and dalbavancin-nonsusceptible with 4-fold, 8-fold, and 128-fold increases in each respective minimum inhibitory concentration (MIC). VAHP-2049 exhibited a modest beta-lactam seesaw effect, with MICs dropping by 1–2 $\log_2$ dilutions. At the time VAHP-2049 was isolated from the patient, the dalbavancin plasma concentration was 24 mg/L. Assuming 99% average protein binding, the circulating unbound concentration was 0.24 mg/L or approximately 0.5 times the MIC of VAHP-2049.

Lipidomics and Membrane Fluidity

Changes in lipid composition and membrane fluidity are illustrated in Supplementary Figure 1. For all lipid classes, VAHP-2049 exhibited decreased levels of long-chain species (≥C33 total carbon number for those with 2 fatty acyl chains: PGs, DGDGs, LysylPGs, PGps, and PAs; ≥C19 for FFAs) (Supplementary Figure 1). Except for LysylPGs, all lipid classes also displayed slightly increased levels in short-chain species (C29–C31). The levels of all species of LysylPGs decreased significantly in VAHP-2049 relative to the parent strain. These changes together result in a more rigid cell membrane in VAHP-2049 as indicated by a higher degree of fluorescence polarity (Supplementary Figure 1).

DISCUSSION

Off-label dalbavancin use has been increasing and is likely fueled by coronavirus disease 2019 (COVID-19)–related pressures to reduce healthcare exposures. This is the first report of a clinically derived strain of dalbavancin-nonsusceptible MRSA with cross-resistance to daptomycin and a mutation in the *walKR* operon similar to the strains we selected for in our in vitro models [2]. We sequenced more than 50 strains evolved under dalbavancin exposure and 75% acquired mutations in *walKR*-associated genes and most exhibited cross-resistance to vancomycin and daptomycin, suggesting that clinicians should exercise caution when considering dalbavancin for invasive MRSA infections. Since dalbavancin inhibits peptidoglycan cross-linking like vancomycin and anchors in the membrane somewhat like daptomycin, it may be well suited to select for cross-resistance to both agents [12].

WalKR is an essential 2-component signal transduction system that regulates autolytic activity, which is known to affect glycopeptide susceptibility, and coordinates aspects of cell division [13, 14]. Previous studies have linked *walKR* mutations with vancomycin and daptomycin nonsusceptibility, but of the 2 case reports of dalbavancin resistance with genomic data available neither directly implicated *walKR* [4, 5]. We performed comprehensive lipidomic analysis to understand the link between dalbavancin and daptomycin cross-resistance. The role of WalKR in modulating lipid metabolism in *S. aureus* has not yet been reported, but in *Staphylococcus pneumoniae*, *walR* induction led to increased expression of fatty acid synthesis genes (eg, *fabKDGF* and *accABC*) [15, 16]. Thus, it is plausible that a reduced-function *walK* mutation could downregulate fatty acid synthesis, consistent with the observed

decreases in long-chain fatty acids and lipids observed in this study. The reduction in PGs, which daptomycin targets, coupled with increased membrane rigidity may best explain cross-resistance to daptomycin in VAHP-2049. Mutations in *walK* have also been found to lead to changes to amino acid, purine, and pyrimidine metabolism, but the contribution of these changes to the antimicrobial susceptibility remains unclear [17].

Conclusions

This case validates in vitro findings suggesting that clinical exposures of dalbavancin can readily select for cross-resistance to vancomycin and daptomycin via *walK* mutation associated with alterations in cell membrane metabolism.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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