Failure of High-Dose Daptomycin for Bacteremia Caused by Daptomycin-Susceptible *Enterococcus faecium* Harboring LiaSR Substitutions

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High-dose daptomycin (DAP) therapy failed in a neutropenic patient with bloodstream infection caused by a DAP-susceptible *Enterococcus faecium* (minimum inhibitory concentration, 3 µg/mL) harboring genetic changes associated with DAP resistance, with persistent bacteremia and selection of additional resistances. Daptomycin monotherapy should be used cautiously against DAP-susceptible *E. faecium* strains with minimum inhibitory concentrations >2 µg/mL.

Keywords. daptomycin; *Enterococcus faecium*; resistance; treatment failure.

The Clinical and Laboratory Standards Institute breakpoint for daptomycin (DAP) in enterococci (4 µg/mL) has been questioned since DAP-susceptible (DAP-S) *Enterococcus faecium* isolates with DAP minimum inhibitory concentrations (MICs) close to the breakpoint (3–4 µg/mL) harbor mutations associated with DAP resistance [1]. Substitutions in LiaFSR, a 3-component regulatory system predicted to orchestrate the cell envelope stress response, have been previously implicated in

DAP resistance [2–4]. Concomitant substitutions in LiaS (T120A) and LiaR (W73C), the putative histidine kinase and response regulator (LiaR) of the system, respectively, are the most common changes observed in *E. faecium* isolates with higher DAP MICs [5]. Moreover, a deletion of a single isoleucine in LiaF (predicted to interact with LiaSR) was sufficient to abolish DAP bactericidal activity against a DAP-S clinical isolate of (MIC, 4 µg/mL) *E. faecalis* [6]. However, clinical correlations corresponding to these in vitro findings are lacking. In this report, we describe a neutropenic patient who had bacteremia with an initially DAP-S *E. faecium* (MIC, 3 µg/mL) harboring T120A and W73C substitutions in LiaS and LiaR, respectively, in whom high-dose DAP therapy (8 mg/kg/d) failed, resulting in recurrent bloodstream infection with a DAP-R isolate, contributing to the patient's death.

CASE REPORT

The patient was a 19-year-old-man with relapsed/refractory B-cell acute lymphocytic leukemia diagnosed in 2011. Treatment was started with fludarabine, cytarabine, idarubicin, and granulocyte colony-stimulating factor and was complicated by febrile neutropenia and *Clostridium difficile* colitis. Catheter-associated bacteremia later developed with a DAP-S (MIC, 3 µg/mL) vancomycin-resistant *E. faecium* (VRE), and treatment was started with DAP (8 mg/kg/d) plus gentamicin (3 mg/kg/d). The patient's weight was 72 kg, and his estimated renal function was normal (plasma creatinine level, 0.9 mg/dL). He remained bacteremic after 7 days of therapy despite catheter removal and no evidence of endovascular foci. Subsequent blood cultures yielded a VRE with a DAP MIC of 16 µg/mL. Therapy was switched to quinupristin–dalfopristin, tigecycline, and linezolid with initial clearing of the infection after 5 days; antibiotics were continued for 14 days. Two weeks after the end of this regimen, VRE bacteremia recurred. Quinupristin–dalfopristin, tigecycline, and linezolid were restarted with blood clearance after 4 days; therapy was continued for 10 days, and the patient was discharged with a prescription for oral linezolid. He was readmitted 10 days later, still during linezolid therapy, with new VRE bacteremia with DAP and linezolid MICs of 24 and 96 µg/mL, respectively. Treatment was started with a combination of tigecycline, DAP (10 mg/kg/d), and ampicillin. An *E. faecium* isolate recovered 36 hours after admission exhibited DAP and linezolid MICs of 256 and 64 µg/mL, respectively. The infection ultimately cleared after 6 days of therapy, and the
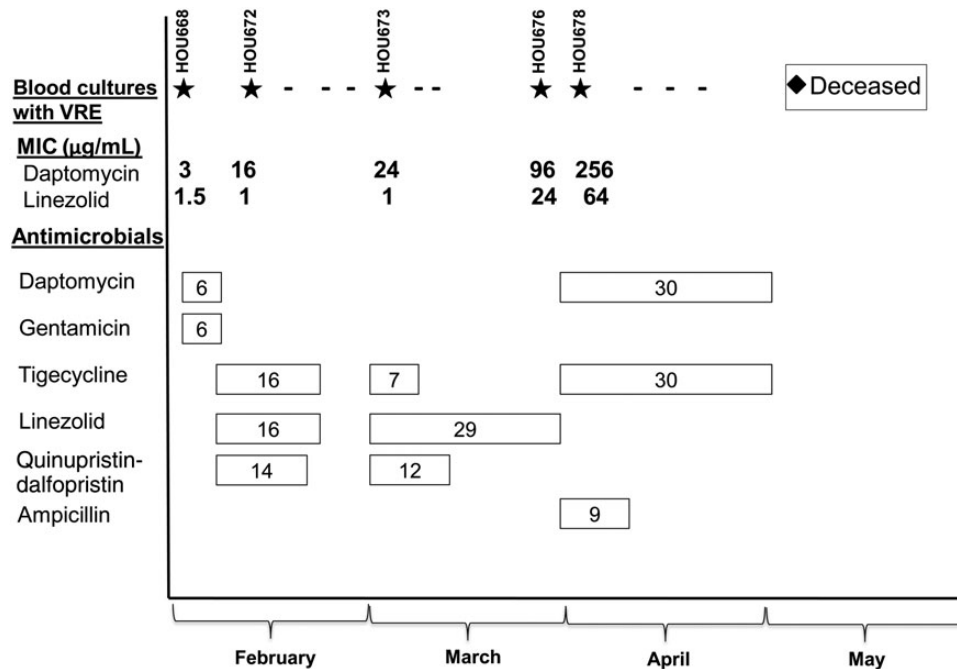
Figure 1. Summary of the patient's clinical course. Numbers in boxes represent days of treatment with the antibiotic listed to the left; stars, positive blood cultures with vancomycin-resistant *Enterococcus faecium* (VRE); numbers above stars, isolate numbers; and dashes, sterile blood cultures. Abbreviation: MIC, minimum inhibitory concentration.

patient remained nonbacteremic for the rest of the hospital stay. Subsequently, an invasive mold pneumonia and *Candida guillermondii* bloodstream infection developed, and the patient died in May (Figure 1).

METHODS

We characterized 5 VRE isolates sequentially recovered from the bloodstream of the patient during an 8-week period. Susceptibilities were determined with Etest (bioMérieux), and genetic relatedness was assessed with pulsed-field gel electrophoresis [7]. We sequenced the following genes previously associated with DAP resistance in enterococci: *liaFSR* (see above); *yycFG*, encoding a 2-component regulatory system involved in cell wall homeostasis; and *cls*, encoding a cardiolipin synthase [3]. Mutations were defined as a nucleotide change resulting in an amino acid substitution not present in any other DAP-S *E. faecium* genomes.

We assessed DAP in vitro bactericidal activity against the first VRE isolate recovered from the bloodstream (HOU668; DAP MIC, 3 µg/mL), using time-kill curves; the control was *E. faecium* TX16, a DAP-S isolate (MIC, 2 µg/mL) whose genome is sequenced and closed. Time-kill assays with and without DAP (5-fold the MIC) were performed as described elsewhere [6]. Bactericidal activity was defined as a decrease of ≥3 $\log_{10}$ in bacterial counts at 24 hours.

Boron-dipyrromethene (BODIPY)-labeled DAP was used to evaluate DAP interaction with the surface of HOU668 (initial isolate) and the last 2 DAP-R isolates (HOU676 and HOU678; DAP MIC, 96 and >256 µg/mL, respectively) and compared with TX16, as described elsewhere [8]. Cell membrane (CM) phospholipids (PLs) were extracted and identified as described elsewhere [9], by means of 2-dimensional thin-layer chromatography with control plates. Results are reported as the relative proportions of the 4 major CM PLs detected. Data are presented as percentages (±SD) from 3 independent experiments.

RESULTS AND DISCUSSION

The 5 bloodstream VRE isolates exhibited an identical pulsed-field gel electrophoresis pattern, indicating they were derivatives of the first isolate (HOU668) (data not shown). HOU668 was DAP susceptible (MIC, 3 µg/mL) yet already harbored the previously described LiaS (T120A) and LiaR (W73C) substitutions without any changes in YYcFG or Cls. A progressive increase in the DAP MIC was observed up to >256 µg/mL (Figure 1), and the above-mentioned LiaSR substitutions were maintained in all isolates. Daptomycin was not bactericidal against HOU668; DAP concentrations of 5-fold MIC only decreased bacterial counts to <1 $\log_{10}$ colony forming units/mL at 24 hours. In contrast, DAP was bactericidal against *E. faecium* TX16 (DAP MIC, 2 µg/mL; Figure 2A). The initial clinical failure resulted in the
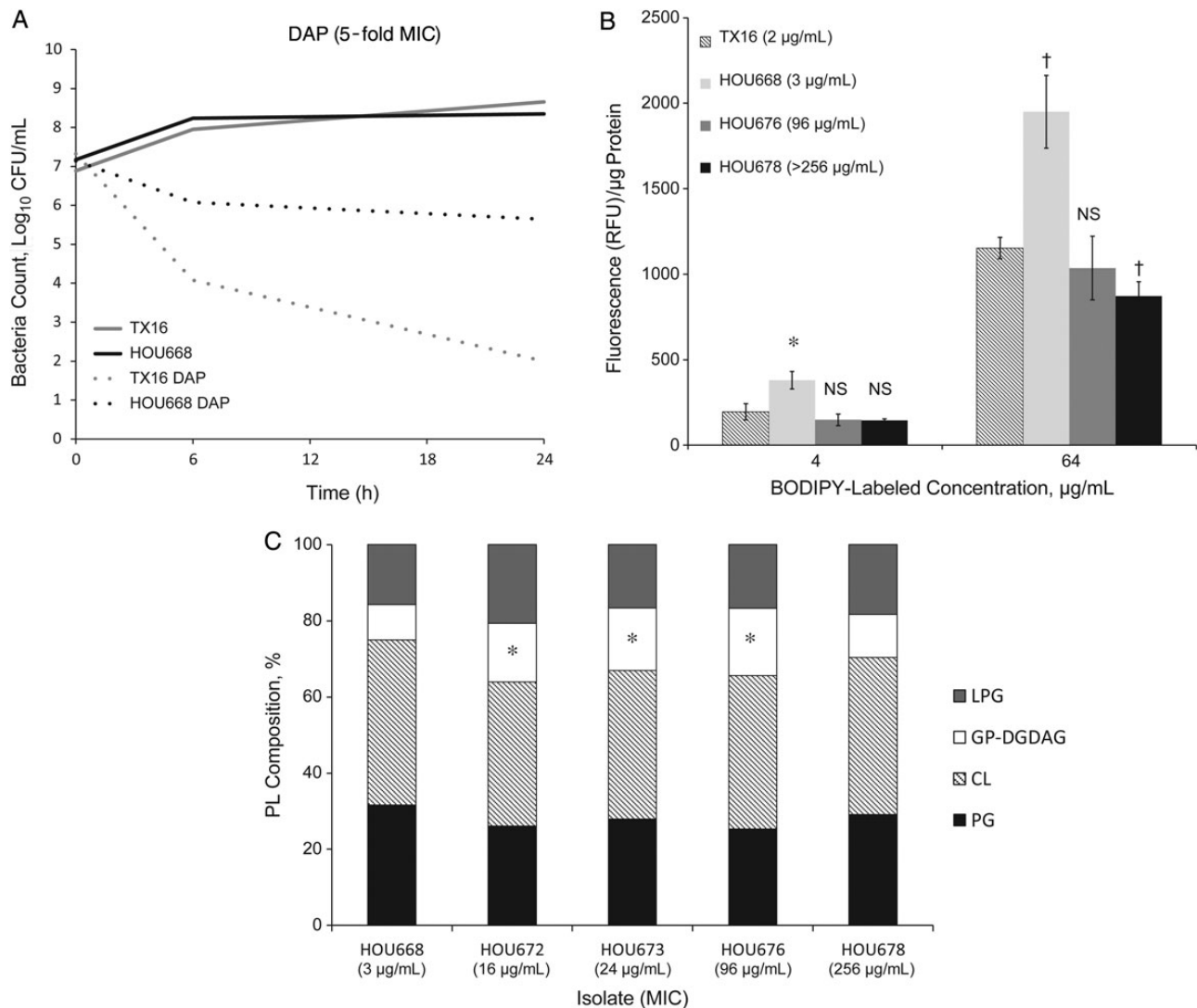
Figure 2. *A,* Time-kill assays with daptomycin (DAP) at 5-fold the minimum inhibitory concentration (MIC). Time-kill curves were obtained using brain heart infusion broth supplemented with calcium to a final concentration of 50 mg/mL. HOU668 was the first vancomycin-resistant *Enterococcus faecium* (VRE) isolated from the patient's bloodstream; it was DAP susceptible (MIC, 3 µg/mL) and harbored amino acid substitutions in LiaS and LiaR. The control strain was TX16, a DAP-susceptible isolate (MIC, 2 µg/mL) whose genome is entirely sequenced and closed and has no mutations related to DAP resistance. *B,* BODIPY-labeled DAP staining of *E. faecium* strains. Cells were treated with BODIPY-labeled DAP, and fluorescence was normalized to cell protein content. Fluorescence intensities were compared with those of *E. faecium* TX16 (see above). *$P < .05$; †$P < .001$. *C,* Phospholipid (PL) composition of the cell membrane of the *E. faecium* strain set. Bacteria were grown in brain-heart infusion and PLs were separated using 2-dimensional thin-layer chromatography. All comparisons were performed using as a reference, HOU668, the only DAP-susceptible isolate obtained from the patient. Results are presented as the percentage of the total cell membrane composition that each PL represents. *$P < .05$; Abbreviations: BODIPY, boron-dipyrromethene; CFU, colony-forming units; CL, cardiolipin; DAP, daptomycin; GP-DGDAG, glycerolphospho-diglycodiacylglycerol; LPG, lysyl-phosphatidylglycerol; MIC, minimum inhibitory concentration; NS, not significant; PG, phosphatidylglycerol; PL, phospholipid; RFU, relative fluorescence units.

use of several antibiotics, with subsequent development of linezolid resistance, precluding the use of 2 front-line anti-VRE antibiotics. After the initial clinical failure, the patient was given 7 antimicrobials, which may have predisposed him to the fungal infection that led to his death.

On the basis of this case, as well as our previous findings [1], we propose that 2 µg/mL may be a better cutoff for DAP

nonsusceptibility in *E. faecium*. Data in staphylococci and enterococci suggest that the combination of DAP and β-lactams can yield in vitro synergism and therapeutic success against DAP-R isolates [10]. Recently, using whole-genome analysis of *E. faecium* isolates, we confirmed that changes in LiaFSR were common in DAP-R strains and in isolates with MICs close to the breakpoint, as in the present case [5]. We also

demonstrated that the combination of DAP plus ampicillin was synergistic against an *E. faecium* with LiaFSR changes but not against an isolate with changes in other genetic pathways associated with DAP resistance [5]. Therefore, although the combination of DAP with a β-lactam may be an interesting option for the treatment of *E. faecium* isolates with DAP MICs between 3 and 4 µg/mL, this combination may not be effective in isolates with genetic changes not involving the LiaFSR system. Our results also suggest that minimal changes in MIC may influence the therapeutic outcome; therefore, Etest could be a better tool to identify *E. faecium* isolates that may respond poorly to therapy.

Interestingly, in this patient, a combination of ampicillin, DAP, and tigecycline resulted in sustained clearance of bacteremia of the DAP and linezolid-resistant VRE. The combination of DAP plus tigecycline has been successfully used in 2 refractory cases of VRE endocarditis and in 1 case of nosocomial meningitis [11–13]. Tigecycline is a bacteriostatic antibiotic with in vitro activity against VRE that may be an interesting option as part of combination therapy for recalcitrant enterococcal infections. In our patient, all isolates retained susceptibility to tigecycline (MIC range, 0.125–0.19 µg/mL).

To gain insights into the mechanism of DAP resistance, we characterized DAP surface binding and CM PL profiles. The binding of DAP to the CM of DAP-S HOU668 (MIC, 3 µg/mL) was significantly higher than to the control strain TX16 (MIC, 2 µg/mL; Figure 2B). Thus, the lack of bactericidal activity against HOU668 was not due to repulsion of the antibiotic from the cell surface. These findings are in line with our previous data, suggesting that diversion of the antibiotic away from its main site of action (the septum) may play a role in withstanding the DAP attack in HOU668 [8]. However, as the DAP MIC increased, repulsion from the cell surface seemed to play a more predominant role in preventing the antibiotic from reaching its target (Figure 2B).

We previously showed that emergence of DAP resistance in *E. faecium* was associated with a significant decrease in the CM content of phosphatidylglycerol and an increase in the negatively charged PL, glycerolphospho-diglycodiacylglycerol (GP-DGDAG). In the current strain set, there was no statistically significant difference in phosphatidylglycerol content between the initial DAP-S isolate and all the other DAP-R derivatives. Although an increase in GP-DGDAG was observed in the first 3 derivatives of HOU668 (DAP MICs, 16, 24, and 96 µg/mL), no change was documented in the last isolate (MIC, >256 µg/mL), suggesting that a complex turnover of PLs occurs during the development of DAP resistance (Figure 2C).

We provide evidence that DAP therapy failed in a DAP-S *E. faecium* with an MIC close to the breakpoint and harboring LiaSR substitutions, a genotype associated with poor bactericidal activity in vitro and DAP resistance, despite the use of high doses. In addition, our data open the possibility of using genotypic information to tailor therapy against recalcitrant VRE infections. Until then, combination therapy with DAP plus another agent (or agents), such as those used in this case (ampicillin plus tigecycline), should be strongly considered for the treatment of deep-seated infections due to *E. faecium* with a DAP MIC >2 µg/mL.

Notes

Potential conflicts of interest. All authors: No potential conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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