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Linezolid-Resistant *Enterococcus faecalis* Isolated from a Cord Blood Transplant Recipient

Imad Dibo,[1] Satish K. Pillai,[2] Howard S. Gold,[2] Maria R. Baer,[1] Meir Wetzler,[1] James L. Slack,[1] Peggy A. Hazamy,[1] Donna Ball,[1] Chiu Bin Hsiao,[1] Philip L. McCarthy, Jr.,[1] and Brahm H. Segal[1]*

Department of Medicine, Roswell Park Cancer Institute, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, New York,[1] and Department of Medicine, Division of Infectious Diseases, Beth Israel Deaconess Medical Center, Harvard School of Medicine, Boston, Massachusetts[2]

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We report a linezolid-resistant *Enterococcus faecalis* infection in a cord blood stem cell transplant recipient previously treated with linezolid for bloodstream infections by vancomycin-resistant enterococci. Sequencing showed a G2576U mutation in the 23S rRNA gene. Because of the important niche of linezolid in cancer treatment, linezolid-resistant *E. faecalis* is noteworthy.

CASE REPORT

A 37-year-old woman underwent allogeneic cord blood stem cell transplantation as therapy for acute myeloid leukemia in second remission at Roswell Park Cancer Institute (Buffalo, N.Y.). The pretransplant course was complicated by invasive pulmonary filamentous fungal infection morphologically consistent with an *Aspergillus* species. The patient had a complete response to voriconazole. The posttransplant course was complicated by prolonged neutropenia and acute graft-versus-host disease (GVHD) involving skin and bowel, necessitating a prolonged course of high-dose corticosteroid therapy. Multiple courses of antibiotics were administered for persistent neutropenic fever and polymicrobial bacteremia with *Klebsiella pneumoniae* and a viridans group streptococcus. Six weeks after transplant, vancomycin-resistant *Enterococcus gallinarum* bacteremia developed and was treated initially with quinupristin-dalfopristin and then with linezolid due to hepatic toxicity associated with quinupristin-dalfopristin. Three months after transplant, the patient developed sternal chest pain and a computed tomography scan showed a lytic lesion in the sternum. A culture of a needle aspirate grew vancomycin-resistant *Enterococcus faecium*. The patient was treated with a 7-week course of linezolid for sternal osteomyelitis. A transthoracic echocardiogram did not show evidence of vegetations; a transesophageal echocardiogram was not performed because of mucositis associated with persistent neutropenia.

Two weeks after linezolid therapy was stopped, bilateral pulmonary infiltrates developed, and the patient required mechanical ventilation. Bronchoalveolar lavage fluid grew linezolid-resistant *Enterococcus faecalis* and cytomegalovirus. Treatment with foscarnet and intravenous immunoglobulin was initiated for presumed cytomegalovirus pneumonitis. The *E. faecalis* isolate was considered to be a respiratory colonizer unrelated to the respiratory failure.

Further complications developed, including pancolitis associated with GVHD, colonic pseudo-obstruction, progressive liver failure (probably related to GVHD and medication-related toxicity), azotemia, and *Candida glabrata* cholecystitis requiring percutaneous drainage. In the setting of refractory multiorgan disease, aggressive therapy was withdrawn and the patient died.

Susceptibility testing. Despite resistance to linezolid, the *E. faecalis* isolate was susceptible to vancomycin, penicillins, and aminoglycosides (Table 1). The isolate showed quinupristin-dalfopristin resistance, which is a characteristic of *E. faecalis*. Testing for susceptibility to linezolid and quinupristin-dalfopristin was performed by disk diffusion according to NCCLS guidelines (19). The zone diameter for linezolid was 7 mm after 24 h of incubation (the cutoff diameter for susceptibility is ≥21 mm). MICs of the other antimicrobial agents were determined with the Vitek system.

Sequencing the 23S rRNA gene. Genomic DNA was extracted from an overnight culture of the linezolid-resistant *E. faecalis* by using previously published methods (20). On the basis of sequence data from GenBank for the *E. faecalis* 23S rRNA gene (accession no. AJ295306), primers to amplify the domain V region were designed and optimized with Oligo primer analysis software (version 5.0; National Biosciences). The upper primer sequence was 5′-GCGGTCGCCTCCTAA AAG-3′, and the lower primer sequence was 5′-ATCCCGGT CCTCTCGTACTA-3′. Following amplification with *Taq* DNA polymerase (Promega), the PCR product was separated by agarose gel electrophoresis and purified (QIAGEN). Sequencing of the PCR product was performed by standard dideoxynucleotide methods (Molecular Biology Core Facility, Dana-Farber Cancer Center, Boston, Mass.). Sequence data were analyzed with EditSeq (DNASTAR), MegAlign (DNASTAR), and Chromas 1.45 (Technelysium) software. The se-

* Corresponding author. Mailing address: SUNY at Buffalo, Division of Infectious Diseases, Roswell Park Cancer Institute, Buffalo, NY 14263. Phone: (716) 845-5721. Fax: (716) 845-3423. E-mail: brahm.segal@roswellpark.org.

TABLE 1. Antibiotic susceptibility of linezolid-resistant *E. faecalis* isolate

Antibiotic	MIC (μg/ml)	Interpretation[a]
Vancomycin	≤0.5	S
Ampicillin	0.25	S
Penicillin	1.0	S
Levofloxacin	≥8	R
Chloramphenicol	≥32	R
Nitrofurantoin	≤32	S
Gentamicin	<500	Syn, S
Streptomycin	<2,000	Syn, S
Linezolid		R
Quinupristin-dalfopristin		R

[a] S, susceptible; R, resistant; Syn, synergy expected.

quenced portion of the 23S rRNA gene contained the G2576U mutation (*Escherichia coli* numbering) previously reported to be associated with linezolid resistance (13, 14, 16, 21, 22).

Discussion. We report a case of linezolid-resistant *E. faecalis* isolated from a cord blood stem cell transplant recipient previously treated with a prolonged course of linezolid for infections caused by VRE. Sequence analysis showed that the 23S rRNA gene had a G2576U mutation, which is known to be associated with linezolid resistance. Linezolid is the first of a new class of synthetic antimicrobial agents, the oxazolidinones, to be approved for use in patients. Linezolid meets an important need based on its activity against virtually all clinically relevant gram-positive pathogens, including oxacillin-resistant *Staphylococcus aureus*, penicillin-resistant pneumococci, and vancomycin-resistant enterococci (VRE) (17). Linezolid is active against *E. faecalis* and *E. faecium*, whereas quinupristin-dalfopristin is active against *E. faecium* isolates but not against *E. faecalis* isolates (8).Linezolid binds to ribosomal 50S subunits, most likely within domain V within the 23S rRNA peptidyl transferase, with a secondary interaction with the 30S subunit (5). In the laboratory, strains of linezolid-resistant enterococci have been generated by serial passage of clinical VRE isolates by using doubling dilutions of linezolid; linezolid resistance was associated with mutations in the domain V region of the 23S rRNA gene (21). Clinical infections by linezolid-resistant *Enterococcus* species have been reported at individual centers (2, 9, 11, 13) but have been rare in the United States and internationally (3, 4, 6). The true frequency of linezolid-resistant enterococcal infections may be underestimated, because many laboratories do not routinely test enterococccal isolates for linezolid susceptibility.

Nosocomial VRE bloodstream infections are an important cause of morbidity in patients with cancer (10, 23). Outbreaks of VRE occur as a consequence of contact transmission and have been documented in cancer centers (10). Hospitalized patients with cancer may be particularly prone to VRE colonization and bloodstream infection based on multiple coexisting risk factors: the use of cephalosporins (18), the use of vancomycin and agents with anaerobic activity (7), prolonged hospitalization, neutropenia (12), and chemotherapy-induced mucositis (15). Because of the important niche of linezolid in therapy for VRE, the identification of even a single linezolid-resistant enterococcal isolate in a cancer center is noteworthy.

Cord blood transplant recipients have a higher risk of infections than other allograft recipients during the early transplant period because of slower myeloid engraftment. A significant frequency of opportunistic infections in cord blood transplant recipients after day 100 in the setting of GVHD has also been noted (1).

The linezolid-resistant *E. faecalis* isolate obtained from our patient was susceptible to vancomycin, penicillins, and aminoglycosides (Table 1). Linezolid-resistant enterococci are usually resistant to vancomycin and to other antimicrobial agents, though rare cases of clinical enterococcal isolates that are linezolid resistant but vancomycin susceptible have been identified (13). This finding may reflect the fact that linezolid is commonly used to treat VRE infections.

Since the initial culture of the linezolid-resistant *E. faecalis* in February 2002, no additional case of linezolid-resistant *Enterococcus* species has been identified at Roswell Park Cancer Institute. We therefore believe that in this patient linezolid-resistant enterococcal infection developed from a spontaneous G2576U mutation under the selective pressure of prolonged linezolid therapy and was not acquired from an external source. The linezolid-resistant *E. faecalis* isolate was recovered 15 days after treatment with linezolid was stopped, supporting the observation by Prystowsky et al. (21) that G2576U is a stable mutation that likely does not adversely affect bacterial viability.

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