

Life-Threatening Infection Caused by Daptomycin-Resistant *Corynebacterium jeikeium* in a Neutropenic Patient[∇]

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Received 4 March 2009/Returned for modification 7 April 2009/Accepted 24 April 2009

Daptomycin is a novel lipopeptide antibiotic agent approved for the treatment of gram-positive life-threatening infections. Here we report, for the first time, the isolation of a highly daptomycin-resistant strain of *Corynebacterium jeikeium* causing a life-threatening infection in a neutropenic patient undergoing cord blood transplantation for secondary acute myeloid leukemia.

CASE REPORT

A 46-year-old man with an uneventful past medical history was diagnosed with acute lymphoblastic leukemia in early 2007. He received a first allogeneic stem cell transplant from a matched unrelated donor after successful induction and consolidation therapy. Early after transplantation, he presented with secondary acute myeloid leukemia and was induced with cyclophosphamide and clofarabine, leading to prolonged neutropenia. Without neutrophil recovery, he proceeded to a second allogeneic stem cell transplantation using a double cord blood source. Since vancomycin-resistant *Enterococcus faecium* (VRE) was detected in earlier stool samples of this patient, two further stool samples were taken after admission to the transplant unit and the presence of VRE was confirmed. Due to this finding, the prior systemic antibiotic chemotherapy and persistent neutropenia, and continuing fever, we treated the patient with a combination of ceftazidime and tigecycline (Fig. 1).

After conditioning chemotherapy was initiated, the antibiotic treatment was changed to meropenem and fosfomycin due to recurring febrile temperatures. While fever continued for 2 days, three blood cultures taken on day –6 (before the second transplant) were found to be positive for vancomycin-sensitive *E. faecium* and *Staphylococcus haemolyticus*. As both isolates showed susceptibility to daptomycin, the antibiotic regimen was changed to daptomycin and ceftazidime (Fig. 1). The patient recovered from febrile temperatures and received a double cord blood allograft on day 0 (8 January 2008). Blood cultures taken from each of the four central venous catheter lines on days –1 and +1 remained sterile. The clinical course was again complicated on day +3 by increasing C-reactive protein (CRP) levels and fever. The antibiotic regimen was

therefore changed to meropenem, fosfomycin, and ciprofloxacin, leading to a short-term decrease of CRP levels and clinical symptoms.

Due to recurrent febrile temperatures, the past infectious history, and positive results for gram-positive rods in two out of four blood cultures taken on day +5, fosfomycin was substituted for daptomycin on day +8. Four further aerobic and anaerobic blood cultures, taken on day +8 and incubated at 37°C in a BacT/Alert 3D instrument (bioMérieux, Marcy-l'Étoile, France), were found to be positive for gram-positive rods after 24 h of cultivation. Subculture testing for another 24 h at 37°C in a 5% CO₂-enriched atmosphere revealed small, gray, catalase-positive colonies on chocolate and blood agar. The organism was subsequently identified as *Corynebacterium jeikeium* ($P = 0.963$) by using an API Coryne V3.0 kit (bioMérieux, Marcy-l'Étoile, France). This result was confirmed by PCR amplification of a 414-bp segment of the 16S rRNA gene using primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 519r (5'-GWATTACCGCGGCKGCTG-3') as described in reference 9, followed by standard Sanger sequencing using fluorescently labeled dideoxynucleotides and an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA) for fragment separation and detection, respectively. The isolated strain was designated *C. jeikeium* strain 1087. Antimicrobial susceptibility testing was performed on Mueller-Hinton agar plates supplemented with 5% sheep blood at 37°C in a 5% CO₂-enriched atmosphere using Etest strips (AB Biodisk, Solna, Sweden) as described in reference 6. Testing revealed daptomycin resistance in all strains isolated, as indicated by MIC values of >256 µg/ml (Table 1). Also tested were VRE strain 381, isolated from an earlier stool sample of the same patient; a type strain of *C. jeikeium* (DSMZ 7171), kindly provided by the German Resource Centre for Biological Material (DSMZ, Braunschweig, Germany); and another *C. jeikeium* isolate, obtained from a different patient who was suffering from bacterial endocarditis (*C. jeikeium* strain 957). According to the manufacturer's instructions, strains *E. faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 29213) were included as technical controls for the daptomycin Etest (Table 1). As can be further seen in Table 1, in contrast to *C. jeikeium* strain 1087, all other strains tested were susceptible to dapto-

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[∇] Published ahead of print on 6 May 2009.

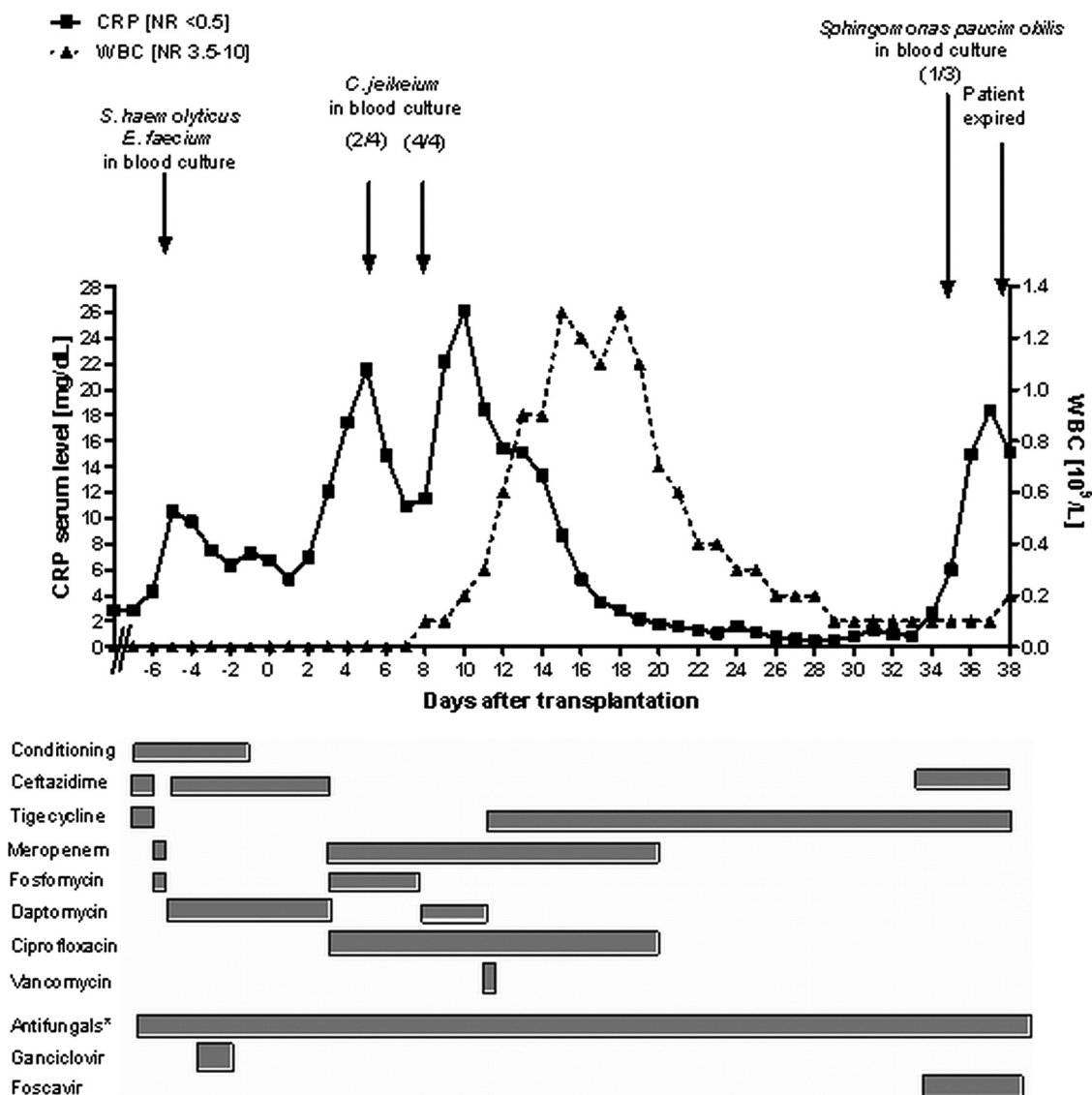


FIG. 1. Schematic diagram of clinical course at transplant. Ratios in parentheses show the number of cultures positive for the indicated organism versus the number tested. Dosing of antibiotic drugs was as follows: ceftazidime, 2 g intravenously (i.v.) three times daily; tigecycline, 50 mg i.v. twice daily after 100 mg i.v. as a loading dose; meropenem, 1 g i.v. three times daily; fosfomycin, 3 g i.v. three times daily; daptomycin, 500 mg i.v. once daily for three days [7.5 mg/kg of body weight] followed by 350 mg i.v. once daily [5 mg/kg]; ciprofloxacin, 500 mg orally and 400 mg i.v., respectively, twice daily; vancomycin, 1 g i.v. twice daily with pharmacomonitoring. *, Antifungal therapy included one of the following: voriconazole, caspofungin, posaconazole, or liposomal amphotericin B. Abbreviations: WBC, white blood cell count; NR, normal range; L, liter.

mycin, with MIC values of <0.25 µg/ml. However, *C. jeikeium* strain 1087 was clearly susceptible to vancomycin and tigecycline.

Due to the unexpected finding of a daptomycin-resistant *C. jeikeium* strain in the blood samples and the septic presentation, with neutropenia, daptomycin was replaced with tigecycline on day +11. The patient recovered clinically and remained free of fever, and CRP serum levels declined almost to the normal range during the next weeks. Unfortunately, this infectious episode appeared to be associated with a secondary graft failure. The patient never achieved full neutrophil recovery, and from day +34 on, CRP levels reaccelerated despite broad antibacterial, antifungal, and antiviral treatment, probably due to progressive pulmonary infection. Consequently, the

patient died from multiorgan failure 38 days after transplant (Fig. 1). Autopsy was refused by his family.

The increasing number of hospital-acquired infections caused by gram-positive bacteria resistant to a large variety of antibiotics, such as methicillin-resistant *S. aureus* (10, 16) or VRE (1), has been countered by the development of novel antibiotic compounds (2). Among them, the lipopeptide daptomycin has recently been approved by the FDA for the treatment of complicated methicillin-resistant *S. aureus* skin infections (15) and was successfully used for the treatment of bacteremia (11) and external-ventricular-drain-associated ven-

TABLE 1. Results of antimicrobial susceptibility testing using Etests on Mueller-Hinton agar supplemented with 5% sheep blood, done in duplicates

Drug	MIC ($\mu\text{g/ml}$) ^a for:					
	Patient isolate		Control strain			
	<i>C. jeikeium</i> strain 1087	VRE strain 381	<i>C. jeikeium</i> strain 957	<i>C. jeikeium</i> DSMZ 7171	<i>E. faecalis</i> ATCC 29212	<i>S. aureus</i> ATCC 29213
AMP	>256	ND	0.75	>256	ND	ND
CIP	>32	ND	0.125	0.125	ND	ND
CLI	>256	ND	1.5	12	ND	ND
DAP	>256	2	0.25	0.125	3	0.016
ERY	>256	ND	0.094	2	ND	ND
GEN	>256	ND	0.094	>256	ND	ND
LVX	>32	ND	0.094	0.19	ND	ND
MEM	2	ND	0.38	0.38	ND	ND
PEN	>32	ND	2	>32	ND	ND
TIG	0.094	>32	0.094	1	ND	ND
VAN	0.50	24	0.50	0.75	ND	ND

^a AMP, ampicillin; CIP, ciprofloxacin; CLI, clindamycin; DAP, daptomycin; ERY, erythromycin; GEN, gentamicin; LVX, levofloxacin; MEM, meropenem; PEN, penicillin; TIG, tigecycline; VAN, vancomycin; ND, not determined.

triculitis (4) with VRE. Although numerous previous studies showed a broad susceptibility of many different gram-positive pathogens to daptomycin (17), daptomycin-resistant *S. aureus* (14) and *E. faecium* (13) have recently been reported. Here, we describe for the first time the isolation of a highly daptomycin-resistant *C. jeikeium* strain isolated from a neutropenic patient undergoing cord blood transplantation.

Colonization of such indwelling devices as central venous catheters by *C. jeikeium* is a common problem among immunocompromised patients and may be associated with consecutive bacteremia and septic infections (7). Accordingly, *C. jeikeium* has already been considered as an opportunistic pathogen which easily acquires resistance to various antibiotic agents (19). In the present case, the changing antibiotic combinations over more than 2 weeks (from days -7 to +8 [Fig. 1]) might have caused a reduction of the commensal microbial flora and a concomitant growth of *C. jeikeium*, finally resulting in selection of the daptomycin-resistant strain. While high-level resistance to daptomycin has not been described in corynebacteria so far (8, 17), and the genetic basis for daptomycin resistance also remains to be elucidated, daptomycin's activity against *C. jeikeium* is known to be inferior to its activity against staphylococci, suggesting the presence of intrinsic factors contributing to lower susceptibility (20). Although we cannot exclude the possibility that the genetic determinant conferring resistance to daptomycin in *C. jeikeium* resides on a low-copy-number plasmid, our attempts to isolate extrachromosomal DNA failed. This suggests that resistance to daptomycin may be encoded on the bacterial chromosome, as is known to be the case for *S. aureus* and *E. faecium*. However, the individual mechanism of resistance may differ among gram-positive bacterial species. For example, mutations in the lysylphosphatidylglycerol synthetase gene (*mprF*) were shown to contribute to daptomycin resistance in *S. aureus* (5). In contrast, genomic comparisons between *C. jeikeium* K411 (18) and *S. aureus* MW 2 (3) showed no *mprF* homology. Furthermore, we wish to emphasize that in the present case, dap-

tomycin resistance was clearly independent of vancomycin resistance in *C. jeikeium* (Table 1). This is in line with previous results for the putative mechanisms underlying daptomycin resistance in *E. faecium* (12). Based on these findings, daptomycin resistance in *C. jeikeium* may be a consequence of the accumulation of numerous genetic events. It may also involve a set of nonspecific mechanisms, such as changes in the charge, permeability, or composition of the corynebacterial cell wall.

As resistance to daptomycin in gram-positive rods still appears to be a rather rare and therefore unexpected phenomenon, we strongly encourage daptomycin susceptibility testing, particularly for high-risk patients with life-threatening infections.

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