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Native Valve Endocarditis Caused by *Corynebacterium striatum* with Heterogeneous High-Level Daptomycin Resistance: Collateral Damage from Daptomycin Therapy?

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We describe a patient who developed *Corynebacterium striatum* native valve endocarditis after receiving two 6-week courses of daptomycin for the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia and osteomyelitis. The organism exhibited *in vitro* heteroresistance to daptomycin, with two subpopulations showing daptomycin susceptibility (MIC of ≤0.094 μg/ml) and high-level resistance to daptomycin (MIC of ≥256 μg/ml). The selection of daptomycin-resistant Gram-positive skin flora with the potential of causing invasive disease may be a concern during prolonged courses of daptomycin.

Corynebacterium striatum is a part of the normal human skin flora and has been implicated in a variety of infections, including pulmonary disease and meningitis. However, infective endocarditis (IE) caused by *Corynebacterium* spp., particularly *C. striatum*, is rare (8). Furthermore, the isolation of *C. striatum* in clinical settings is complicated by its relatively slow growth and difficult identification at the species level (4). *C. striatum* bacteria are generally susceptible to many antibiotics, but multidrug-resistant isolates have been described (11).

Daptomycin is a lipopeptide antibiotic that exhibits potent *in vitro* bactericidal activity against many Gram-positive pathogens, including multidrug-resistant organisms. It is FDA approved for skin and soft tissue infection and bacteremia caused by *Staphylococcus aureus* (3). Nonsusceptibility to daptomycin has been described for *S. aureus*, enterococci, and one isolate of *Corynebacterium jeikeium* (MIC of 256 μg/ml) (14). Heteroresistance has been extensively documented in *S. aureus* with β-lactams and vancomycin (7, 13) but is uncommon with daptomycin. Here, we describe a case of native valve endocarditis and heteroresistance to daptomycin caused by *C. striatum* recovered from a patient who had previously received two prolonged courses of daptomycin for the treatment of methicillin-resistant *S. aureus* (MRSA) bacteremia and osteomyelitis.

A 56-year-old man with type 2 diabetes mellitus and end-stage renal disease was transferred to our hospital with a 1-week history of fever, lethargy, and dyspnea. During the previous year, the patient had received two 6-week courses of daptomycin at 6 mg/kg of body weight for treatment of catheter-related bloodstream infection and osteomyelitis of the left foot caused by MRSA. The last course had been completed 3 weeks before the current admission. The patient had a history of allergy to vancomycin described as acute onset of agitation, anxiety, and shortness of breath soon after initiation of the vancomycin infusion, and on admission to an outside hospital, he was begun on daptomycin at 6 mg/kg intravenously, every 48 h. However, the patient did not respond to therapy and was transferred to our institution. On admission to our hospital, the patient was febrile (39°C), had a blood pressure of 112/72 mmHg, and was tachycardic. The physical examination showed a lethargic man with jugular venous distention and a 3/6 pansystolic murmur at the apex. Examination of the lungs revealed bilateral rales. The laboratory results showed anemia and leukocytosis, and a transesophageal echocardiogram showed a 3- by 4-cm vegetation with abscess formation on the posterior leaflet of the mitral valve and severe mitral regurgitation. The chest X-ray and magnetic resonance imaging of the brain showed bilateral lung infiltrates and bilateral thalamic and posterior occipital lobe infarctions, respectively. *Corynebacterium* spp. bacteria were isolated in four sets of blood cultures, and susceptibility testing revealed MICs of 0.5 μg/ml for vancomycin, 4 μg/ml for gentamicin, 0.004 μg/ml for rifampin, and ≥256 μg/ml for daptomycin. Daptomycin was stopped and telavancin at 10 mg/kg intravenously every 48 h (MIC of 0.19 μg/ml by Etest) was initiated. Repeated blood cultures were negative, and the patient underwent mitral valve replacement. The patient had an uneventful immediate postoperative course and was discharged with intravenous telavancin to a long-term-care facility. Three weeks after discharge from the hospital, the patient was readmitted with severe sepsis, candidemia, and multiorgan failure. Blood cultures remained negative for bacterial pathogens, but the patient expired due to complications of sepsis.

Identification of the organism at the species level and molecular typing were performed using 16S rRNA sequencing and the repetitive-sequence-based PCR (rep-PCR) method using the Diversilab system with the *Corynebacterium* kit (bioMérieux) (5), respectively. Antimicrobial susceptibilities were determined using Etest on Mueller-Hinton medium supplemented with blood at 24 h of incubation. We evaluated the relative net surface charge and depolarization of the cell membrane using a modified cytochrome

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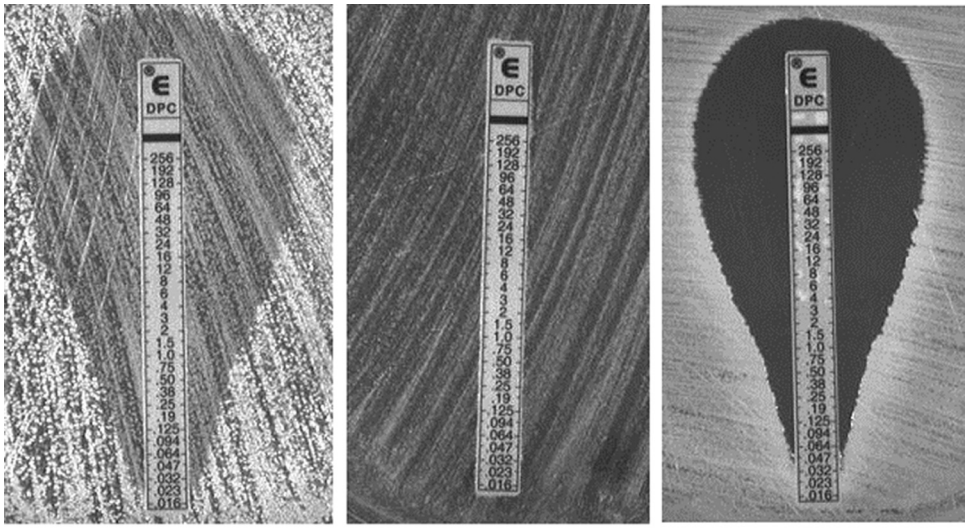

FIG 1 Daptomycin Etest results with *C. striatum* isolated from patient's blood. (A) The Etest showed two subpopulations. The resistant population grew close to the edge of the strip. A second halo of inhibition was observed with colonies growing away from the strip (the susceptible subpopulation). (B) Daptomycin Etest of purified single colonies recovered from the resistant subpopulation; homogeneous growth was observed, exhibiting a daptomycin MIC of ≥256 μg/ml. (C) Daptomycin Etest of purified colonies from the susceptible subpopulation; homogeneous growth was observed, with a daptomycin MIC of 0.094 μg/ml.

c assay and the membrane potential-sensitive fluorescent dye DiSC3 (5) (daptomycin concentration 0.125 to 32 μg/ml), respectively, as previously described (1). The percentage of fluorescence loss was calculated, setting the isopropanol control as 100% fluorescence change and buffer as 0% fluorescence change. Values are expressed as means plus or minus standard deviations, and differences were analyzed with the Student *t* test. A *P* value of less than 0.05 was considered statistically significant.

The infecting organism was identified as *C. striatum*. When the daptomycin MICs were determined using the Etest methodology, two halos of inhibition were clearly distinguished (Fig. 1A). The first subpopulation had colonies which grew next to the Etest strip at concentrations of ≥256 μg/ml. The second halo of inhibition harbored colonies that only grew at low concentrations of daptomycin (Fig. 1A). In an attempt to separate these two subpopulations, bacterial growths from the edge of the Etest strip at higher concentrations (resistant subpopulation) and beyond the halo of inhibition (susceptible subpopulation) were restreaked for single colonies which were then retested for daptomycin susceptibility by Etest. We observed homogeneous growth of a resistant subpopulation with a daptomycin MIC of ≥256 μg/ml (Fig. 1B) and a susceptible subpopulation exhibiting a daptomycin MIC of ≤0.094 μg/ml (Fig. 1C), suggesting that the two subpopulations were probably selected by the presence of the antibiotic. Genotyping using the rep-PCR methodology indicated that both subpopulations had identical banding patterns (not shown), confirming that they were the same strain. Susceptibilities to other antibiotics remained the same, except for penicillin (Table 1).

The target of daptomycin is the bacterial cell membrane, leading to bacterial cell death (3). Development of daptomycin resistance during therapy has been well characterized in *S. aureus* and, to a lesser extent, in enterococci (1, 15). The resistance phenotype has been associated, in some strains of *S. aureus* and *Enterococcus faecalis*, with changes in cell surface charge and alteration in the ability of daptomycin to depolarize the cell membrane (1, 15). Daptomycin depolarized the membrane of the susceptible isolate in a concentration-dependent manner at concentrations above 2 μg/ml. Interestingly, this effect by daptomycin was abolished in the *C. striatum*-resistant isolate (at concentrations up to 32 μg/ml, $P < 0.001$) (Fig. 2). However, depolarization was not associated with changes in the cell surface charge as determined by a cytochrome *c* assay, which did not show a significant difference between the amount of unbound cytochrome *c* between the susceptible and resistant isolates (not shown).

Apart from *C. diphtheriae*, other members of the *Corynebacterium* genus are considered organisms of low pathogenicity and, in general, are regarded as commensals of the skin flora but capable of causing clinically significant infections, mostly in immunocompromised patients (8). IE caused by *C. striatum* has been reported in patients with prosthetic valves and history of intravenous drug abuse and has been associated rarely with native valve IE (2).

Daptomycin is a cyclic lipopeptide antibiotic with activity

TABLE 1 Antimicrobial susceptibility testing of daptomycin-susceptible (Coryne-S) and daptomycin-resistant (Coryne-R) *C. striatum*

Antibiotic	MIC (μg/ml)[a]	
	Coryne-S	Coryne-R
Ampicillin	2	2
Cefotaxime	>32	>32
Daptomycin	≤0.094	≥256
Gentamicin	12	12
Levofloxacin	>32	>32
Linezolid	0.125	0.125
Penicillin	1.5	0.75
Telavancin	0.19	0.19
Vancomycin	0.5	0.5

[a] MICs were determined by the Etest method on Mueller-Hinton blood agar. Coryne-S, daptomycin-susceptible *C. striatum*; Coryne-R, daptomycin-resistant *C. striatum*.

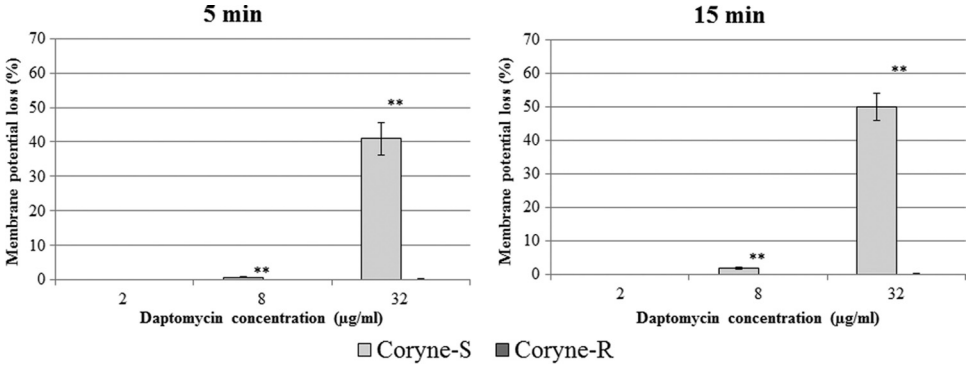

FIG 2 Concentration-dependent daptomycin-induced cell membrane potential changes in daptomycin-susceptible and -resistant *C. striatum* populations. The data shown are after 5 and 15 min of exposure to the antibiotic. Uptake of DiSC3(5) was compared between daptomycin-susceptible (Coryne-S) and daptomycin-resistant (Coryne-R) *C. striatum* populations. Error bars show standard deviations. **, $P < 0.001$.

against Gram-positive bacteria that has been used in clinical practice since 2003. Daptomycin was initially used in our patient for the treatment of MRSA bacteremia and osteomyelitis since the patient had a reported history of vancomycin allergy. Indeed, our patient received at least two prolonged courses of daptomycin (6 mg/kg), the last one completed a few weeks before the diagnosis of *C. striatum* IE. The history of daptomycin therapy strongly suggests that resistance was selected upon exposure to daptomycin, but it is unknown when resistance developed. One scenario is that resistance emerged in a commensal organism which then caused endocarditis and the susceptible variant represents an *in vitro* revertant to wild type. Another possibility is that infection caused by a susceptible organism occurred prior to one of the daptomycin courses but was not recognized and that the resistant variant was then selected upon further exposure to daptomycin. To the best of our knowledge, this is the first documented case of *C. striatum* native valve IE exhibiting daptomycin resistance. Interestingly, the only other case of daptomycin-resistant *Corynebacterium* spp. is a *C. jeikeium* isolate recovered from the bloodstream of a neutropenic patient who underwent cord blood transplantation for secondary acute myeloid leukemia (14). This isolate also exhibited high-level resistance to daptomycin (MIC > 256 μg/ml) and was recovered after daptomycin therapy for *Staphylococcus haemolyticus* and vancomycin-sensitive *Enterococcus faecium* bacteremia (14). Thus, it is very plausible that the use of prolonged courses of daptomycin may also exert pressure on the Gram-positive skin commensal flora and select for high daptomycin resistance in organisms such as *Corynebacterium* spp. with the potential of causing severe disease, such as IE. Of note, a dose of 6 mg/kg was used in this case; it is unknown if the use of higher doses (8 to 10 mg/kg) for the treatment of severe MRSA infections (9) may prevent the emergence of resistance in the commensal skin flora.

Another interesting observation from this case is that the organism was fully susceptible to telavancin and microbiological eradication was achieved with this antibiotic. Telavancin is a lipoglycopeptide that binds to peptidoglycan precursors but also interferes with cell membrane function (6). The therapeutic response suggests that the mechanism of daptomycin resistance in this isolate does not interfere with the activity of telavancin. Telavancin has been tested in a murine model of endocarditis (10) and in at least in one report of a patient with endocarditis (12), suggesting that this antibiotic may have clinical utility in the treatment of infective endocarditis.

In summary, we describe the emergence of high-level heteroresistance to daptomycin in *C. striatum* from a patient who underwent prolonged therapy with the antibiotic. Our findings suggest that clinicians should be aware of the risk of selecting daptomycin-resistant Gram-positive skin commensal organisms with the ability of causing invasive disease during prolonged courses of daptomycin.

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