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Rapid Emergence of Daptomycin Resistance in Clinical Isolates of *Corynebacterium striatum*... A Cautionary Tale

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Abstract

Purpose—The objective of this study was to investigate the observation of daptomycin resistance in *Corynebacterium striatum*, both *in vivo* and *in vitro*. We describe a case of *C. striatum* bacteremia in a patient with a left ventricular assist device (LVAD); the initial isolate recovered was daptomycin susceptible with a minimum inhibitory concentration (MIC) of 0.125 µg/ml. Two months later, and after daptomycin therapy, the individual became bacteremic with an isolate of *C. striatum* with a daptomycin MIC of >256 µg/ml.

Methods—To study the prevalence of daptomycin resistance in *C. striatum*, clinical isolates of *C. striatum* were grown in broth culture containing daptomycin to investigate emergence of resistance to this antimicrobial. Molecular typing was used to evaluate serial isolates from the index patient and the clinical isolates of *C. striatum* we assayed.

Results—*In vitro* analysis of isolates from the index patient and seven of eleven additional *C. striatum* isolates exhibited emergence of high level daptomycin resistance, despite initially demonstrating low MICs to this antimicrobial agent. This phenotype was persistent even after serial subculture in the absence of daptomycin.

Conclusions—Together, these data demonstrate that caution should be taken when using daptomycin to treat high-inoculum infections and/or infections of indwelling medical devices with *C. striatum*. To our knowledge, this is the first report characterizing emergence of daptomycin resistance in *C. striatum*.

Introduction

Daptomycin is a lipopeptide antibiotic used to treat infections caused by Gram-positive bacteria; it functions by inserting its lipophilic tail into the bacterial cell membrane in a calcium-dependent manner. After insertion, daptomycin molecules aggregate, cause rapid

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depolarization and loss of membrane potential, leakage of cellular contents, and result in inhibition of RNA, DNA, and protein synthesis, ultimately leading to cell death. It is often considered an antibiotic of last resort and reserved to treat resistant Gram-positive pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) [1-5].

Historically, few *Corynebacterium* spp. were considered to be disease-causing pathogens and when isolated in clinical cultures, they were frequently attributed to normal skin flora and/or culture contaminants. Recent advances in bone marrow and solid organ transplantation and an increasing life expectancy for patients with chronic diseases has led to a rise in the number of immunosuppressed patients. The use of mechanical left ventricular assist devices (LVAD) in patients with advanced heart failure also continues to increase in frequency. LVADs are used as a “bridge to transplant” (weeks-years) and as long term “destination therapy” (years) and over 11,500 patients have been implanted with these devices in the United States [6]. These successes have led to a concomitant increase in serious infectious attributed to organisms that were traditionally considered to be of low virulence, such as *Corynebacterium* spp. and other Gram-positive flora commonly found on the skin [7,8]. Infections of indwelling hardware are difficult to treat because of the patient's lack of a functional immune response, reduced drug bioavailability at the site of infection due to poor vascularization, drug interactions, and often the hardware cannot be removed if infected. Patients with these infections require long courses of antibiotic treatment with a limited armamentarium of antibiotic options [9,10].

Daptomycin resistance has been previously documented for *S. aureus* and enterococci; the mechanisms of resistance are complex and multifactorial. In *S. aureus*, daptomycin resistance has been attributed to single nucleotide polymorphisms (SNPs) in *mprF*, *ycyG*, *ycyH*, *ropB*, and *ropC* genes as well as upregulation of the *dltABCD* operon [11-16]. These are genes involved in cell membrane synthesis and protein translocation across the cell membrane as well as the generalized bacterial stress response. An accumulation of SNPs in these genes results in depolarization and permeabilization of the cell membrane, increased cell wall thickening, and reduced affinity for daptomycin binding which renders the antibiotic less effective for killing these bacteria [17]. Daptomycin resistance in enterococci has been linked with mutations in genes *liaF*, *ycyG*, and *gdpD*, which regulate cell membrane homeostasis, cell membrane phospholipid metabolism, and bacterial stress response [18-21]. Homologues of genes associated with daptomycin resistance in *S. aureus* and enterococci have not yet been identified in *Corynebacterium* spp.

Herein, we describe a patient who developed bacteremia with an isolate of *C. striatum* which was initially susceptible to daptomycin but became resistant while the patient was receiving daptomycin therapy. There are only two case reports in the literature of daptomycin-resistant *Corynebacterium* spp.; an isolate of *C. jeikeium* from a patient who received a hematopoietic stem cell transplant and an isolate of *C. striatum* from a patient on long term daptomycin therapy for native valve endocarditis [22,23]. The objective of our study was to demonstrate that daptomycin resistance can be induced in a high percentage of *Corynebacterium striatum* isolates *in vivo* and *in vitro*.

Case report

A 61-year-old male with a history of ischemic cardiomyopathy underwent LVAD (Heart Mate II, Thoratec, Pleasanton, CA) implantation as a bridge to heart transplantation. Two months after implantation, the patient developed an LVAD pump pocket abscess from which *Staphylococcus epidermidis* was isolated. After incision and drainage of the abscess, the patient received nine weeks of intravenous (IV) ceftriaxone followed by oral cephalexin for suppression; however, three weeks after finishing the IV antibiotic course, the patient presented with fever and fatigue without pain, tenderness, erythema, or purulent drainage around the LVAD driveline exit site. A computed tomography (CT) scan demonstrated a 6.5 × 3.8 × 4.7 cm abscess inferior to the LVAD pump and a smaller fluid collection adjacent to the exit site of the left ventricle conduit. At this time, *C. striatum* was isolated from two sets of blood cultures (VersaTREK, Thermofisher Scientific) obtained by venipuncture from two sites (susceptibility results shown in Table 1, **Isolate 1**). The patient was started on IV vancomycin and two days later intraoperative cultures obtained during debridement of the pump pocket abscess were negative. A transthoracic echocardiogram (TTE) did not reveal valvular or pacemaker lead vegetations. After six-weeks of vancomycin therapy, the patient developed a petechial rash with biopsy results showing superficial lymphocytic vasculitis that was attributed to vancomycin. Subsequently therapy was changed to IV daptomycin (8 mg/kg/day). Seventeen days into the new regimen, the patient presented with fevers and hypotension. Two out of two blood culture sets grew *C. striatum* that was non-susceptible to daptomycin (Table 1, **Isolate 2**). The antimicrobial regimen was changed to linezolid and prior to completion of this course the patient received an orthotopic heart transplant. Intraoperative cultures obtained from the LVAD pocket were negative; however, cultures from an implantable cardioverter-defibrillator (ICD) lead vegetation grew daptomycin-susceptible *C. striatum* (Table 1, **Isolate 3**). Six weeks of IV linezolid were completed after the heart transplant and the patient recovered well as of one year post transplantation.

Methods

Bacterial Isolates

This study characterizes isolates of *C. striatum* that were recovered from clinical cultures at Barnes-Jewish Hospital from February 2012 to April 2013. All isolates were recovered using standard primary culture media including sheep blood agar (Remel) and/or chocolate agar (Remel). Per standard laboratory procedures for identification of *Corynebacterium* spp., all isolates were catalase positive, Gram-positive rods that were identified as *C. striatum* (99% confidence) using the RapID CB Plus System (Remel, Lenexa, KS). The identity of all isolates in our study was confirmed using Bruker Biotyper MALDI-TOF MS (software version 3.0) with all isolates receiving identification scores of ≥ 2.0 corresponding to “acceptable for species-level identification” [24].

Strain Typing

To determine the relatedness of the *C. striatum* isolates, molecular typing was performed by repetitive-sequence-based PCR (repPCR) with primer RW3A, resolved by the Agilent 2100 analyzer (Agilent Technologies, Santa Clara, CA) and analyzed using Diversilab software

(bioMérieux Clinical Diagnostics, Durham, NC) as previously described [25,26]. Isolates with a similarity index of <95% are considered distinct.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility profiles for the case patient's three *C. striatum* isolates (Table 1) were determined using the gradient diffusion method Etest (bioMérieux, Durham, NC) on standard Mueller-Hinton agar containing 5% sheep blood (Remel) incubated in ambient air for 24 to 48 h. Quality control of reagents and medium and interpretation of MIC values were performed according to Clinical and Laboratory Standards Institute (CLSI) standards. For daptomycin and *Corynebacterium* spp., an MIC of ≤ 1 is susceptible and an MIC of >1 is non-susceptible [27]. For ease of discussion, those isolates categorized as daptomycin non-susceptible will be referred to as daptomycin resistant throughout the manuscript.

Assay for Selection of Daptomycin Resistant Isolates

The three isolates recovered from the index patient in addition to twelve clinical isolates of *C. striatum* from unique patient specimens were each inoculated into two five ml tubes of tryptic soy broth at an optical density of a 0.5 McFarland standard. A daptomycin Etest (bioMérieux), was cut in half and fully submerged into one of the broth cultures while the other broth culture served as a growth control. Broth cultures were vortexed for five seconds to facilitate release of daptomycin from the Etest strip. The strip was left in the broth and the cultures were then incubated with constant agitation at 35°C in room air. After 24 hours of incubation, antimicrobial susceptibility testing for daptomycin was performed on the bacteria grown in both broth tubes as described above.

Results

C. striatum isolates recovered from index patient

The index patient developed blood stream infection with a *C. striatum* isolate that was highly resistant to daptomycin subsequent to daptomycin therapy. All isolates from our index patient were susceptible to vancomycin with MICs ranging from 1-2 µg/ml (Table 1). We performed repPCR to assess clonality of the isolates; all three isolates demonstrated a similarity index (SI) of >98.5% to one another (Figure 1A) [26,25].

In vitro selection for daptomycin resistant *C. striatum*

Since the index patient developed infection with a daptomycin-resistant *C. striatum* while on daptomycin therapy, we hypothesized that a large burden of organism and/or prolonged exposure to daptomycin may have caused the organism to become resistant. To test our hypothesis, we designed an *in vitro* assay to expose isolates to daptomycin and query for resistance. Our patient's three *C. striatum* isolates were grown overnight in broth culture that did not contain daptomycin resulted in MICs of 0.125, >256, and 0.125 µg/ml, respectively for patient isolates 1, 2, and 3 (Figure 1B, **top row**). The same isolates all had MICs of >256 µg/ml after overnight incubation in the presence of daptomycin (Figure 1B, **bottom row**). After selection for high-level daptomycin resistance in our patient's *C. striatum* isolates, they were each subcultured daily for ten days on non-selective, 5% sheep blood agar and incubated at 35°C in 5% CO₂. The daptomycin MICs continued to be >256 µg/ml for all

three isolates after ten rounds of subculture, suggesting that the resistant phenotype is sustained even when daptomycin selective pressure is removed.

To determine if rapid emergence of daptomycin resistance is common among *Corynebacterium*, we collected twelve additional clinical isolates of *C. striatum* originating from a variety of specimen types (Table 2). We performed the same *in vitro* experiment, and of the twelve isolates, one was initially resistant to daptomycin and seven became resistant after overnight incubation in broth containing daptomycin. Four isolates remained susceptible to daptomycin (i.e. either they did not grow in the presence of daptomycin or their MIC did not change) (Table 2). All resistant isolates retained high-level resistance to daptomycin after ten rounds of subculture on non-selective media.

There was some variability in the *C. striatum* daptomycin susceptibility testing following our *in vitro* experiment. Although most isolates showed complete resistance to daptomycin (no zone around Etest strip), one isolate from our index patient had a zone of inhibition with pinpoint colonies within the zone (Figure 1B; **bottom, right**). A second clinical isolate, exhibited an MIC of 8 µg/ml after exposure to daptomycin in broth culture.

Strain Typing

RepPCR analysis of the three isolates from our index patient and the twelve clinical isolates of *C. striatum* showed that all but one were >95% similar, suggesting that they are the same strain type. One isolate of *C. striatum* obtained from a blood culture had an SI of <80% to the other isolates tested, and is a distinct strain type.

Discussion

An increasing number of immunosuppressed patients and those with long term indwelling hardware have led to an increase in the number of patients with serious, invasive *Corynebacterium* infections that require prolonged treatment [8,28,7]. Successful treatment of *Corynebacterium* infections with daptomycin has been reported in the literature, and a number of studies have demonstrated *Corynebacterium* spp. possessing low MICs to daptomycin *in vitro* [29-32]. In this report, the index patient developed a daptomycin-resistant isolate of *C. striatum* during treatment with daptomycin. There are only two case reports of daptomycin-resistant *Corynebacterium* in the literature, one isolate of *C. jeikeium* and a second of *C. striatum* [23,22], although since daptomycin susceptibility testing is rarely performed on *Corynebacterium* spp., it is possible that occurrence of this phenomenon is underestimated. Similar to our index patient, in both of the prior case reports, daptomycin-resistant *Corynebacterium* spp. were isolated from blood specimens of patients who had received daptomycin treatment. However, in contrast to our study, there was no recovery of daptomycin-susceptible *Corynebacterium* prior to the recovery of daptomycin-resistant *Corynebacterium* in either case report. Although our index patient received daptomycin treatment specifically for his *C. striatum* infection, both patients reported in the literature were initially treated with daptomycin for infections with other organisms (MRSA; *Enterococcus faecium* and *Staphylococcus haemolyticus*), after which they became infected with *Corynebacterium* spp. This highlights the role of antibiotic

selection for acquisition of daptomycin-resistant *Corynebacterium* spp. infections in patients receiving daptomycin treatment.

Daptomycin resistance is tightly correlated with increased vancomycin MICs in isolates of *S. aureus* [33]. This is thought to be due to the phenotypic phenomenon of cell wall thickening found in vancomycin-intermediate *S. aureus*, and that the thickened cell wall may alter the net charge of the outer membrane rendering daptomycin insertion into the cell membrane less effective. The only report of daptomycin-resistant *Corynebacterium* that assessed cell wall thickness found no difference between daptomycin-sensitive and resistant *Corynebacterium* isolates [34,23]. Daptomycin-resistant *Corynebacterium* isolates from both case reports and our index patient were all susceptible to vancomycin [23,22].

Our *in vitro* experiments demonstrated that a large number of *C. striatum* isolates that are initially susceptible to daptomycin can rapidly acquire high-level resistance when incubated in the presence of daptomycin. The majority of *C. striatum* clinical isolates in which we induced daptomycin resistance *in vitro* were resistant to the highest concentration of daptomycin present on the Etest strip (>256 µg/ml). Of note, our *in vitro* experiment demonstrated decreased turbidity in broth cultures (determined by visual inspection of the broth) containing daptomycin compared to control cultures at four hours of incubation, but this lag in growth disappeared by 24 hours of incubation. The initial delay of growth in broth of the daptomycin-susceptible isolates suggests that one of two mechanisms of resistance may be occurring: a heteroresistant population may be present which is selected as the predominant population in the presence of daptomycin, or a phenotype of inducible resistance may exist. Reports of daptomycin resistance in *S. aureus* and enterococci show an increase in MIC, but not the extreme change demonstrated herein over a very short time interval. For example, reports of enterococci illustrate that mutations of two genes known to be involved in daptomycin resistance increased the MIC from one to twelve µg/ml [18]. Future studies are necessary to determine the mechanism of daptomycin resistance in *Corynebacterium*.

In the current study, daptomycin-resistant *C. striatum* isolates retained their resistant phenotype after subculture on non-selective agar for ten days, indicating that once acquired, daptomycin resistance in *C. striatum* is relatively stable. This differs from reports in *S. aureus*, which demonstrated that to maintain daptomycin resistance, isolates must be subcultured on media containing daptomycin [14].

Many clinical laboratories do not routinely perform antimicrobial susceptibility testing for daptomycin, especially for organisms such as *Corynebacterium* spp. Controversy exists surrounding the optimal method for daptomycin susceptibility testing, and some evaluations have shown variability in results for isolates that are very near to the “resistant” breakpoint [2,35-37]. However, all but one of the isolates in this study that initially demonstrated very low daptomycin MICs (0.25 µg/ml or less) subsequently demonstrated MICs of >256 µg/ml, the highest dilution resolvable using Etest, after exposure to daptomycin. It is highly unlikely that the demonstration of resistance is an artifact of our testing methodology due to the dramatic change in MIC. Additionally, the *in vivo* evidence, treatment failure, supports the result of daptomycin resistance in the *Corynebacterium* strain described herein.

In summary, our data demonstrate just how rapidly *Corynebacterium striatum* can develop daptomycin resistance both *in vivo* and *in vitro*. Since there are several differences between what is known about daptomycin resistance in *S. aureus* and enterococci compared to what we and others have observed in *Corynebacterium* spp., it is possible daptomycin resistance is acquired by a novel mechanism not previously described. This is a serious concern for patients receiving long term daptomycin therapy as these patients are at increased risk for developing serious, opportunistic *Corynebacterium* infections.

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References

1. Gould IM, Miro JM, Rybak MJ. Daptomycin: the role of high-dose and combination therapy for Gram-positive infections. *International journal of antimicrobial agents*. 2013; 42(3):202–210.10.1016/j.ijantimicag.2013.05.005 [PubMed: 23845504]
2. Humphries RM, Pollett S, Sakoulas G. A current perspective on daptomycin for the clinical microbiologist. *Clinical microbiology reviews*. 2013; 26(4):759–780.10.1128/CMR.00030-13 [PubMed: 24092854]
3. Rybak JM, Barber KE, Rybak MJ. Current and prospective treatments for multidrug-resistant gram-positive infections. *Expert opinion on pharmacotherapy*. 2013; 14(14):1919–1932.10.1517/14656566.2013.820276 [PubMed: 23876168]
4. Corona Perez-Cardona PS, Barro Ojeda V, Rodriguez Pardo D, Pigrau Serrallach C, Guerra Farfan E, Amat Mateu C, Flores Sanchez X. Clinical experience with daptomycin for the treatment of patients with knee and hip periprosthetic joint infections. *The Journal of antimicrobial chemotherapy*. 2012; 67(7):1749–1754.10.1093/jac/dks119 [PubMed: 22511636]
5. Durante-Mangoni E, Casillo R, Bernardo M, Caianiello C, Mattucci I, Pinto D, Agrusta F, Caprioli R, Albinetti R, Ragone E, Utili R. High-dose daptomycin for cardiac implantable electronic device-related infective endocarditis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2012; 54(3):347–354.10.1093/cid/cir805 [PubMed: 22100575]
6. INTERMACS. Interagency Registry for Mechanically Assisted Circulatory Support. University of Alabama at Birmingham; 2014. [Accessed February 3 2014]
7. Lee PP, Ferguson DA Jr, Sarubbi FA. *Corynebacterium striatum*: an underappreciated community and nosocomial pathogen. *The Journal of infection*. 2005; 50(4):338–343.10.1016/j.jinf.2004.05.005 [PubMed: 15845432]
8. Funke G, von Graevenitz A, Clarridge JE 3rd, Bernard KA. Clinical microbiology of coryneform bacteria. *Clinical microbiology reviews*. 1997; 10(1):125–159. [PubMed: 8993861]
9. Goldstein EJ, Citron DM, Merriam CV, Warren Y, Tyrrell K, Fernandez HT. In vitro activities of dalbavancin and nine comparator agents against anaerobic gram-positive species and corynebacteria. *Antimicrobial agents and chemotherapy*. 2003; 47(6):1968–1971. [PubMed: 12760876]
10. Goldstein EJ, Citron DM, Merriam CV, Warren YA, Tyrrell KL, Fernandez HT. In vitro activities of daptomycin, vancomycin, quinupristin-dalfopristin, linezolid, and five other antimicrobials against 307 gram-positive anaerobic and 31 *Corynebacterium* clinical isolates. *Antimicrobial agents and chemotherapy*. 2003; 47(1):337–341. [PubMed: 12499210]
11. Ernst CM, Staubitz P, Mishra NN, Yang SJ, Hornig G, Kalbacher H, Bayer AS, Kraus D, Peschel A. The bacterial defensin resistance protein MprF consists of separable domains for lipid lysinylation and antimicrobial peptide repulsion. *PLoS pathogens*. 2009; 5(11):e1000660.10.1371/journal.ppat.1000660 [PubMed: 19915718]

12. Yang SJ, Kreiswirth BN, Sakoulas G, Yeaman MR, Xiong YQ, Sawa A, Bayer AS. Enhanced expression of *dltABCD* is associated with the development of daptomycin nonsusceptibility in a clinical endocarditis isolate of *Staphylococcus aureus*. *The Journal of infectious diseases*. 2009; 200(12):1916–1920.10.1086/648473 [PubMed: 19919306]
13. Mwangi MM, Wu SW, Zhou Y, Sieradzki K, de Lencastre H, Richardson P, Bruce D, Rubin E, Myers E, Siggia ED, Tomasz A. Tracking the in vivo evolution of multidrug resistance in *Staphylococcus aureus* by whole-genome sequencing. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104(22):9451–9456.10.1073/pnas.0609839104 [PubMed: 17517606]
14. Kaatz GW, Lundstrom TS, Seo SM. Mechanisms of daptomycin resistance in *Staphylococcus aureus*. *International journal of antimicrobial agents*. 2006; 28(4):280–287.10.1016/j.ijantimicag.2006.05.030 [PubMed: 16963232]
15. Friedman L, Alder JD, Silverman JA. Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy*. 2006; 50(6): 2137–2145.10.1128/AAC.00039-06 [PubMed: 16723576]
16. Yang SJ, Xiong YQ, Dunman PM, Schrenzel J, Francois P, Peschel A, Bayer AS. Regulation of *mprF* in daptomycin-nonsusceptible *Staphylococcus aureus* strains. *Antimicrobial agents and chemotherapy*. 2009; 53(6):2636–2637.10.1128/AAC.01415-08 [PubMed: 19289517]
17. Bayer AS, Schneider T, Sahl HG. Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. *Annals of the New York Academy of Sciences*. 2013; 1277:139–158.10.1111/j.1749-6632.2012.06819.x [PubMed: 23215859]
18. Arias CA, Panesso D, McGrath DM, Qin X, Mojica MF, Miller C, Diaz L, Tran TT, Rincon S, Barbu EM, Reyes J, Roh JH, Lobos E, Sodergren E, Pasqualini R, Arap W, Quinn JP, Shamoo Y, Murray BE, Weinstock GM. Genetic basis for in vivo daptomycin resistance in enterococci. *The New England journal of medicine*. 2011; 365(10):892–900.10.1056/NEJMoa1011138 [PubMed: 21899450]
19. Munita JM, Tran TT, Diaz L, Panesso D, Reyes J, Murray BE, Arias CA. A *liaF* codon deletion abolishes daptomycin bactericidal activity against vancomycin-resistant *Enterococcus faecalis*. *Antimicrobial agents and chemotherapy*. 2013; 57(6):2831–2833.10.1128/AAC.00021-13 [PubMed: 23507277]
20. Palmer KL, Daniel A, Hardy C, Silverman J, Gilmore MS. Genetic basis for daptomycin resistance in enterococci. *Antimicrobial agents and chemotherapy*. 2011; 55(7):3345–3356.10.1128/AAC.00207-11 [PubMed: 21502617]
21. Munita JM, Panesso D, Diaz L, Tran TT, Reyes J, Wanger A, Murray BE, Arias CA. Correlation between mutations in *liaFSR* of *Enterococcus faecium* and MIC of daptomycin: revisiting daptomycin breakpoints. *Antimicrobial agents and chemotherapy*. 2012; 56(8):4354–4359.10.1128/AAC.00509-12 [PubMed: 22664970]
22. Schoen C, Unzicker C, Stuhler G, Elias J, Einsele H, Grigoleit GU, Abele-Horn M, Mielke S. Life-threatening infection caused by daptomycin-resistant *Corynebacterium jeikeium* in a neutropenic patient. *Journal of clinical microbiology*. 2009; 47(7):2328–2331.10.1128/JCM.00457-09 [PubMed: 19420177]
23. Tran TT, Jaijakul S, Lewis CT, Diaz L, Panesso D, Kaplan HB, Murray BE, Wanger A, Arias CA. Native valve endocarditis caused by *Corynebacterium striatum* with heterogeneous high-level daptomycin resistance: collateral damage from daptomycin therapy? *Antimicrobial agents and chemotherapy*. 2012; 56(6):3461–3464.10.1128/AAC.00046-12 [PubMed: 22450978]
24. McElvania Tekippe E, Shuey S, Winkler DW, Butler MA, Burnham CA. Optimizing identification of clinically relevant Gram-positive organisms by use of the Bruker Biotyper matrix-assisted laser desorption ionization-time of flight mass spectrometry system. *Journal of clinical microbiology*. 2013; 51(5):1421–1427.10.1128/JCM.02680-12 [PubMed: 23426925]
25. Fritz SA, Hogan PG, Camins BC, Ainsworth AJ, Patrick C, Martin MS, Krauss MJ, Rodriguez M, Burnham CA. Mupirocin and chlorhexidine resistance in *Staphylococcus aureus* in patients with community-onset skin and soft tissue infections. *Antimicrobial agents and chemotherapy*. 2013; 57(1):559–568.10.1128/AAC.01633-12 [PubMed: 23147738]
26. El Feghaly RE, Stamm JE, Fritz SA, Burnham CA. Presence of the *bla(Z)* beta-lactamase gene in isolates of *Staphylococcus aureus* that appear penicillin susceptible by conventional phenotypic

- methods. *Diagnostic microbiology and infectious disease*. 2012; 74(4):388–393.10.1016/j.diagmicrobio.2012.07.013 [PubMed: 22959917]
27. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-third Informational Supplement. Clinical and Laboratory Standards Institute; Wayne, PA: 2013. vol M100-S23
 28. Ghidde S, Jiang Y, Hachem R, Chaftari AM, Raad I. Catheter-related *Corynebacterium* bacteremia: should the catheter be removed and vancomycin administered? *European journal of clinical microbiology & infectious diseases* : official publication of the European Society of Clinical Microbiology. 2010; 29(2):153–156.10.1007/s10096-009-0827-0
 29. Fernandez Guerrero ML, Molins A, Rey M, Romero J, Gadea I. Multidrug-resistant *Corynebacterium striatum* endocarditis successfully treated with daptomycin. *International journal of antimicrobial agents*. 2012; 40(4):373–374.10.1016/j.ijantimicag.2012.06.001 [PubMed: 22817918]
 30. Fernandez Guerrero ML, Robles I, Nogales Mdel C, Nuevo D. *Corynebacterium striatum*: an emerging nosocomial drug-resistant endocardial pathogen. *The Journal of heart valve disease*. 2013; 22(3):428–430. [PubMed: 24151771]
 31. Sader HS, Flamm RK, Farrell DJ, Jones RN. Daptomycin Activity against Uncommonly Isolated Streptococcal and Other Gram-Positive Species Groups. *Antimicrobial agents and chemotherapy*. 2013; 57(12):6378–6380.10.1128/AAC.01906-13 [PubMed: 24080651]
 32. Fernandez-Roblas R, Adames H, Martin-de-Hijas NZ, Almeida DG, Gadea I, Esteban J. In vitro activity of tigecycline and 10 other antimicrobials against clinical isolates of the genus *Corynebacterium*. *International journal of antimicrobial agents*. 2009; 33(5):453–455.10.1016/j.ijantimicag.2008.11.001 [PubMed: 19153032]
 33. van Hal SJ, Paterson DL, Gosbell IB. Emergence of daptomycin resistance following vancomycin-unresponsive *Staphylococcus aureus* bacteraemia in a daptomycin-naïve patient--a review of the literature. *European journal of clinical microbiology & infectious diseases* : official publication of the European Society of Clinical Microbiology. 2011; 30(5):603–610.10.1007/s10096-010-1128-3
 34. Montero CI, Stock F, Murray PR. Mechanisms of resistance to daptomycin in *Enterococcus faecium*. *Antimicrobial agents and chemotherapy*. 2008; 52(3):1167–1170.10.1128/AAC.00774-07 [PubMed: 18180351]
 35. Bryant KA, Roberts AL, Rupp ME, Anderson JR, Lyden ER, Fey PD, Van Schooneveld TC. Susceptibility of enterococci to daptomycin is dependent upon testing methodology. *Diagnostic microbiology and infectious disease*. 2013; 76(4):497–501.10.1016/j.diagmicrobio.2013.04.019 [PubMed: 23719086]
 36. Palavecino EL, Burnell JM. False daptomycin-nonsusceptible MIC results by Microscan panel PC 29 relative to Etest results for *Staphylococcus aureus* and enterococci. *Journal of clinical microbiology*. 2013; 51(1):281–283.10.1128/JCM.01721-12 [PubMed: 23077121]
 37. Nakashima H, Takahashi H, Kameko M, Saito H. Daptomycin Etest MICs for methicillin-resistant *Staphylococcus aureus* vary among different media. *Journal of infection and chemotherapy* : official journal of the Japan Society of Chemotherapy. 2012; 18(6):970–972.10.1007/s10156-012-0424-5 [PubMed: 22576751]

Figure 1. Profile of three *C. striatum* isolates from our index patient

A. Dendrogram illustrating the results of repPCR strain typing analysis of the three *C. striatum* isolates recovered from the index patient. All isolates have a SI 95%, illustrating that the infections were caused by a single strain type. **B.** Daptomycin MIC results as evaluated by Etest for the index patient's initial *C. striatum* bloodstream infection (**top, left**), *C. striatum* isolated from the blood stream 66 days later (**top, center**), and *C. striatum* isolated from tissue 78 days after the initial blood stream infection (**top, right**). Each isolate was grown overnight in broth culture in the absence (**top row**) and presence (**bottom row**) of daptomycin.

Table 1

Antibiotic susceptibility profile of three *C. striatum* isolates from the index patient.

Antibiotic	Isolate 1		Isolate 2		Isolate 3	
	MIC (µg/ml)	Interpretation	MIC (µg/ml)	Interpretation	MIC (µg/ml)	Interpretation
Daptomycin	0.125	S	>256	R	0.125	S
Ceftriaxone	>256	R	>256	R	>256	R
Clindamycin	>256	R	>256	R	>256	R
Ciprofloxacin	>32	R	>32	R	>32	R
Linezolid	n/a	n/a	0.25	S	0.5	S
Tetracycline	1	S	0.5	S	1	S
Vancomycin	1	S	2	S	1	S

S=susceptible; R=resistant; n/a=antimicrobial susceptibility testing not performed

Isolate 1 was isolated from blood (day 1); isolate 2 was isolated from blood 66 days later; isolate 3 was isolated from tissue 78 days after the initial *C. striatum* isolate was isolated from blood.

Table 2

In vitro emergence of daptomycin resistance in clinical isolates of *C. striatum*.

Isolate #	Specimen Source	Initial Daptomycin MIC (µg/ml)	Interpretation	MIC after Incubation with Daptomycin (µg/ml)	Interpretation
1	bone, hip	0.064	S	>256	R
2	sputum [#]	0.125	S	0.125	S
3	bone, tibia	0.125	S	>256	R
4	bone, hip	0.064	S	>256	R
5	blood	0.125	S	8	R
6	blood	0.125	S	>256	R
7	blood	0.25	S	NG	S
8	blood	0.125	S	>256	R
9	blood	>256	R	>256	R
10	wound, ear	0.064	S	NG	S
11	blood	0.25	S	NG	S
12	blood	0.125	S	>256	R

S=susceptible; R=resistant; NG=no growth

[#] Daptomycin would not be used to treat infections of the respiratory tract.

All *C. striatum* isolates were isolated from unique patients.