J Infect Chemother 25 (2019) 906–908

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Case Report

Bacteremia due to high-level daptomycin-resistant *Corynebacterium striatum*: A case report with genetic investigation☆

Hideharu Hagiya [a, *], Keigo Kimura [b], Hideo Okuno [a], Shigeto Hamaguchi [a], Daiichi Morii [a], Hisao Yoshida [a], Tomomi Mitsui [b], Isao Nishi [b], Kazunori Tomono [a]

[a] Division of Infection Control and Prevention, Osaka University Hospital, Japan
[b] Laboratory for Clinical Investigation, Osaka University Hospital, Japan

ARTICLE INFO

Article history:
Received 18 February 2019
Received in revised form
5 April 2019
Accepted 10 April 2019
Available online 14 May 2019

Keywords:
Antimicrobial resistance
Corynebacterium
Daptomycin

ABSTRACT

Corynebacterium striatum, generally considered an opportunistic organism in humans, has recently been known to develop high-level daptomycin resistance (HLDR) shortly after drug exposure. To date, however, only several such clinical isolates have been described in the literature and clinical background of the resistant pathogen remains to be elucidated. Here, we report a case involving a *C. striatum* strain with HLDR harboring novel nucleotide mutations, together with a review of the relevant literature. To the best of our knowledge, this is the first well-investigated clinical report from Japan including a genetic investigation. Considering the rapid emergence of HLDR *C. striatum in vitro* experiment, there could be a number of underreporting cases. Scrupulous attention is required when administering daptomycin for the treatment of *C. striatum* infections, even if the organism has initially exhibited susceptibility.

1. Introduction

Daptomycin (DAP) represents a unique class of natural cyclic lipopeptide antibiotics that exert bactericidal activity against both stationary- and log-phase Gram-positive bacterial pathogens. This antibiotic exhibits good pharmacokinetic properties [1] and has been highly recommended in treatment guidelines for methicillin-resistant *Staphylococcus aureus* infections [2]. However, reports have already described the emergence of DAP-non-susceptible *S. aureus* strains both *in vitro* [3] and *in vivo* [4–6]. Moreover, increasing numbers of the literature report emergences of high-level DAP resistance (HLDR) in viridians group streptococci [7] and *Enterococcus faecium* [8].

Corynebacterium species, opportunistic organisms colonizing the human skin, have recently been recognized as the causes of various infections [9]. As organisms potentially show multidrug-resistance to both oral and intravenous antibiotics [10–12], DAP is considered a major agent for the treatment of *Corynebacterium* infections.

Since the first report of the clinical emergence of HLDR in *Corynebacterium jeikeium* in 2009 [13], several such cases have been described in the literature [14–16]. Comprehensive studies of the genomic, lipidomic, and biochemical aspects of DAP have proven that the antimicrobial activity depends on the membrane phosphatidylglycerol concentration [17,18]. According to the literature, a single mutation in phosphatidylglycerol synthase (*pgsA2*), which removes phosphatidylglycerol from the bacterial membrane, can induce the HLDR phenotype [17,18]. We herein report another case of HLDR in a strain of *C. striatum* with a genetic investigation. To our knowledge, this is the first such case reported in a Japanese clinical setting.

2. Case

A 68-year-old man who had undergone a simultaneous pancreas and kidney transplantation procedure consequent to type 1 diabetes mellitus 5 years earlier was hospitalized because of a routine follow-up examination. While hospitalized, the patient complained of lower right abdominal pain at the site of the transplanted graft. A laboratory examination revealed elevated levels of C-reactive protein (7.18 mg/dL), serum pancreatic amylase (647 U/L), and lipase (104 U/L). We diagnosed the patient with chronic graft rejection and administered a 3-day course of

☆ All authors meet the ICMJE authorship criteria.

* Corresponding author. Division of Infection Control and Prevention, Osaka University Hospital, 2-15 Yamadaoka, Suita, Osaka 565-0871, Japan.
E-mail address: highgear@hp-infect.med.osaka-u.ac.jp (H. Hagiya).

https://doi.org/10.1016/j.jiac.2019.04.009
1341-321X/

methylprednisolone (500 mg per day) and piperacillin/tazobactam. His symptoms and laboratory values promptly improved. However, he soon experienced an exacerbation of the abdominal pain, which was accompanied by an elevation of the pancreatic enzyme levels. Another course of high-dose methylprednisolone therapy was administered, and the antimicrobial treatment was switched to a combination of meropenem, micafungin, and gancyclovil.

Despite these changes, the patient subsequently developed black stool and the right lower abdominal tenderness worsened. Contrast-enhanced computed tomography (CECT) revealed an intraperitoneal low-density lesion in the peri-duodenal adipose tissue suggestive of abscess formation. As a result of percutaneous puncture of the abscess, a wide variety of pathogens, including methicillin-resistant *Staphylococcus aureus*, *Enterococcus* species, *Candida* species, and *Corynebacterium* species, were detected in the sample. DAP therapy (250 mg [3.7 mg/kg] per day) was added on, but his condition and laboratory data did not improve after 1 week. A follow-up CECT newly revealed intraperitoneal free air, and an emergent operation revealed the collapse of the transplanted duodenum, which was then resected with the transplanted pancreas.

Twelve days after DAP initiation, the patient developed a high fever. *C. striatum* was isolated from a blood culture examination (MALDI Biotyper, Bruker Daltonics Inc., Billerica, MA, USA) and found to be susceptible (MIC [μg/mL], minimum inhibitory concentration) to vancomycin (0.5), trimethoprim/sulfamethoxazole (1), and rifampicin (≤1), but resistant to penicillin (>4), cefotaxime (>4), ceftriaxone (>4), cefepime (>2), and meropenem (>2) (MICroFAST 7J, Beckman Coulter, Brea, CA USA). Because this organism induced bacteremia under the administration of DAP, we examined its MIC level using Etest and Mueller Hinton E Agar (bioMérieux, Marcy-l'Étoile, FRANCE). Consequently, the isolated *C. striatum* strain was found to exhibit HLDR (MIC: >256 μg/mL). Following a switch to a 4-week course of intravenous vancomycin, the patient eventually recovered.

3. Bacterial analysis

To clarity the resistant mechanism, we performed a sequencing analysis to detect a mutation in *pgsA2*, which encodes phosphatidylglycerol synthase, being known as a gene responsible for DAP resistance [17,18]. We constructed an original primer set for *pgsA2* (Forward: 5′-CAG AGA CTC GCA AAA CGC CA-3′ and Reverse: 5′-TGC AAT GTA CTT CAT GCC CC-3′) and subjected bacterial DNA to a PCR analysis using the following conditions: initial denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 30 sec, annealing at 61 °C for 30 sec, and extension at 72 °C for 1 min; and final extension at 72 °C for 5 min. The PCR products were purified and subsequently sequenced using a BigDye™ Terminator v3.1 Cycle Sequencing Kit and 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

The sequence data (599 nucleotides), deposited in DDBJ Sequence Database with accession number of LC462282, were compared to the sequence of the reference strain, WP1a, in the literature [17]. Consequently, we detected several nucleotide mutations in our sample that led to 5 predicted amino acid substitutions: A65V, D95K, K96H, A97X, and L98X (Table 1). Of these, the deletion of four nucleotides (AGAT) starting at position 282 yielded stop codons at amino acids 97 and 98, causing a crucial inactivating mutation in this isolate.

4. Discussion

We have described a clinical isolate of *C. striatum* with HLDR that was isolated from a patient after a 12-day course of DAP therapy. A sequencing analysis of *pgsA2* encoding phosphatidylglycerol synthase revealed multiple loss-of-function point mutations. As the patient had not previously received DAP therapy, we assume that HLDR emerged in this *C. striatum* strain within 12 days after treatment initiation.

We reviewed the MEDLINE database for previous reports of the clinical emergence of HLDR in *Corynebacterium* species (Table 2). Notably, 6 cases, including ours, have been described since the initial report in 2009 [13–16]. Three of the six cases involved prosthetic device infections, and the others were associated with refractory infections (febrile neutropenia, endocarditis, and intra-abdominal abscess). In all cases, the pathogens were isolated from blood samples. *Corynebacterium jeikeium* was isolated in one case; all other isolates were *C. striatum*. All the isolates exhibited a

Table 1
Amino acid substitutions identified in our isolate relative to a reference strain.

Reference isolate: WP1a
MAGRISPVNDSQAQQKAAAASDVNPNVSNWNLPNVLTSLRILFIPVFAWLVLADHEWWAFGLFVALMITDKLDGDIARSRGLITNFGKIADPIADKALMTAAFVCLNIIGVLPVWVTVVIL
VREMAGRISPVNDSQAQQKAAAASDVNPNVSNWNLPNVLTSLRILFIPVFAWLVLADHEWWAFGLFVALMITDKLDGDIARSRGLITNFGKIADPIADKALMTAAFVCLNIIGVLPVWV
TVVILVREFGITLWRMALLRQGKVVPASKGGKLKTVLQTIAVAMYLCPLPSWMDIPTFAVMLAAVVVTVVTGVQYLIDGRKQNSFGITLWRMALLRQGKVVPASKGGKLKTVLQTIAVA
MYLCPLPSWMDIPTFAVMLAAVVVTVVTGVQYLIDGRKQNS
Isolate: the present case
MAGRISPVNDSQAQQKAAAASDVNPNVSNWNLPNVLTSLRILFIPVFAWLVLADHEWWAFGLFV**V**LMITDKLDGDIARSRGLITNFGKIADPIA
KHXX*RLHSSVSISSVCCLCGXRWLSWCASSALPCGAWLCCAKARWCQHLRAASXRLFSKPXLLPCTCARYRAGWISLLLXLCSWQLWSPWSPVCSTSLTVGSRIA*

The upper and lower boxes present the amino acid sequences of the reference strain, WP1a [18], and the isolate from the present case, respectively. The substitutions identified in our isolate are expressed in bold, underlined text (5 positions). The amino acid sequences after the nonsense mutations are indicated in italic font with a dotted underline.

Table 2
Summary of the reported cases of high-level daptomycin (DAP) resistance in *Corynebacterium* species.

Year	Patient age (years) and sex	Infections	Sample	Isolate	MIC level of DAP	Preceding DAP administration	Reference
2009	46, man	Febrile neutropenia	Blood	*Corynebacterium jeikeium*	>256 μg/mL	After 8 days of DAP at 7.5 mg/kg	[14]
2012	56, man	Native valve endocarditis	Blood	*Corynebacterium striatum*	≥256 μg/mL	After 3 weeks of two 6-week courses DAP at 6 mg/kg	[15]
2014	61, man	LVAD infection	Blood	*Corynebacterium striatum*	>256 μg/mL	After 17 days of DAP at 8 mg/kg	[16]
2016	67, man	LVAD infection	Blood	*Corynebacterium striatum*	>256 μg/mL	After 16 days of DAP at 6 mg/kg	[17]
2016	67, man	LVAD infection	Blood	*Corynebacterium striatum*	>256 μg/mL	After 3 months of DAP at 6 mg/kg	[17]
2018	68, man	Intraperitoneal abscess	Blood	*Corynebacterium striatum*	>256 μg/mL	After 12 days of DAP at 3.7 mg/kg	present case

MIC, minimum inhibitory concentration; LVAD, left ventricular-assist device.
In all cases, the MIC values for DAP were examined using Etest.

DAP MIC level ≥256 μg/mL. The duration of DAP administration varied from approximately 1 week to several months, and a DAP dose of ≥6 mg/kg was administered in all but the present case. Notably, *C. striatum* isolates with HLDR were shown to develop at a high frequency (7/11 tested isolates [15], or 48/480 tested isolates [12]) after an overnight *in vitro* incubation with DAP. These findings strongly suggest that clinicians should monitor the emergence of such highly resistant pathogenic strains.

A loss of function in *pgsA2* was previously shown to be responsible for the development of *Corynebacterium* isolates exhibiting HLDR. Recent studies reported amino acid changes in *pgsA2*, such as G147D [17] and an F174 frameshift [18]. In our case, we identified five predicted amino acid changes, including stop codons (A97X and L98X). To our knowledge, our report describes the first amino acid replacements that may be associated with the development of HLDR in *Corynebacterium* species.

In conclusion, we have described a case of bacteremia attributed to *C. striatum* with HLDR, which was isolated after 12 days of DAP treatment. Additionally, our sequencing analysis has revealed the genetic background of this isolate. Currently, DAP is considered a last-resort antibiotic for the treatment of Gram-positive infections. This rapid evolution of a highly DAP-resistant organism highlights the significance of appropriate antibiotic use and the importance of routine clinical screening for HLDR among *Corynebacterium* species.

Conflicts of interest

None to report.

References

[1] Heidary M, Khosravi AD, Khoshnood S, Nasiri MJ, Soleimani S, Goudarzi M. Daptomycin. J Antimicrob Chemother 2018;73:1–11.

[2] Liu C, Bayer A, Cosgrove SE, Daum RS, Fridkin SK, Gorwitz RJ, et al. Clinical practice guidelines by the infectious diseases society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. Clin Infect Dis 2011;52:285–92.

[3] Rose WE, Rybak MJ, Tsuji BT, Kaatz GW, Sakoulas G. Correlation of vancomycin and daptomycin susceptibility in *Staphylococcus aureus* in reference to accessory gene regulator (agr) polymorphism and function. J Antimicrob Chemother 2007;59:1190–3.

[4] Fowler VG, Boucher HW, Corey GR, Abrutyn E, Karchmer AW, Rupp ME, et al. Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. N Engl J Med 2006;355:653–65.

[5] Sader HS, Moet GJ, Farrell DJ, Jones RN. Antimicrobial susceptibility of daptomycin and comparator agents tested against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci: trend analysis of a 6-year period in US medical centers (2005–2010). Diagn Microbiol Infect Dis 2011;70:412–6.

[6] Matono T, Hayakawa K, Hirai R, Tanimura A, Yamamoto K, Fujiya Y, et al. Emergence of a daptomycin-non-susceptible *Enterococcus faecium* strain that encodes mutations in DNA repair genes after high-dose daptomycin therapy. BMC Res Notes 2016;9:197.

[7] Akins RL, Katz BD, Monahan C, Alexander D. Characterization of high-level daptomycin resistance in viridans group streptococci developed upon in vitro exposure to daptomycin. Antimicrob Agents Chemother 2015;59:2102–12.

[8] Humphries RM, Kelesidis T, Tewhey R, Rose WE, Schork N, Nizet V, et al. Genotypic and phenotypic evaluation of the evolution of high-level daptomycin nonsusceptibility in vancomycin-resistant *Enterococcus faecium*. Antimicrob Agents Chemother 2012;56:6051–3.

[9] Wong KY, Chan YC, Wong CY. *Corynebacterium striatum* as an emerging pathogen. J Hosp Infect 2010;76:371–2.

[10] Martínez-Martínez L, Pascual A, Bernard K, Suárez AI. Antimicrobial susceptibility pattern of *Corynebacterium striatum*. Antimicrob Agents Chemother 1996;40:2671–2.

[11] Hahn WO, Werth BJ, Butler-Wu SM, Rakita RM. Multidrug-resistant *Corynebacterium striatum* associated with increased use of parenteral antimicrobial drugs. Emerg Infect Dis 2016;22:1908–14.

[12] McMullen AR, Anderson N, Wallace MA, Shupe A, Burnham C-AD. When good bugs go bad: epidemiology and antimicrobial resistance profiles of *Corynebacterium striatum*, an emerging multidrug resistant, opportunistic pathogen. Antimicrob Agents Chemother 2017;61:e01111–7.

[13] Schoen C, Unzicker C, Stuhler G, Elias J, Einsele H, Grigoleit GU, et al. Life-threatening infection caused by daptomycin-resistant *Corynebacterium jeikeium* in a neutropenic patient. J Clin Microbiol 2009;47:2328–31.

[14] Tran TT, Jaijakul S, Lewis CT, Diaz L, Panesso D, Kaplan HB, et al. Native valve endocarditis caused by *Corynebacterium striatum* with heterogeneous high-level daptomycin resistance: collateral damage from daptomycin therapy? Antimicrob Agents Chemother 2012;56:3461–4.

[15] McElvania TeKippe E, Thomas BS, Ewald GA, Lawrence SJ, Burnham CAD. Rapid emergence of daptomycin resistance in clinical isolates of *Corynebacterium striatum*… a cautionary tale. Eur J Clin Microbiol Infect Dis 2014;33:2199–205.

[16] Werth BJ, Hahn WO, Butler-Wu SM, Rakita RM. Emergence of high-level daptomycin resistance in *Corynebacterium striatum* in two patients with left ventricular assist device infections. Microb Drug Resist 2016;22:233–7.

[17] Goldner NK, Bulow C, Cho K, Wallace M, Hsu F-F, Patti GJ, et al. Mechanism of high-level daptomycin resistance in *Corynebacterium striatum*. mSphere 2018;3. e00371–18.

[18] Hines KM, Waalkes A, Penewit K, Holmes EA, Salipante SJ, Werth BJ, et al. Characterization of the mechanisms of daptomycin resistance among Gram-positive bacterial pathogens by multidimensional lipidomics. mSphere 2017;2. e00492–17.