Original Articles

Clinical and microbiological characteristics of high-level daptomycin-resistant *Corynebacterium* species: A systematic scoping review

Running title: Daptomycin-resistant *Corynebacterium*

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Authors' contributions

SF and HH conceived the study; ST, KG, SF, and KI performed the microbiological testing; SF and HA evaluated and selected articles; SF drafted the manuscript; HH revised the manuscript; OM and FO supervised the study. All authors interpreted the results and gave final approval to the submitted manuscript.

Abstract

Introduction: *Corynebacterium* species potentially develop high-level daptomycin resistance (HLDR) shortly after daptomycin (DAP) administration. We aimed to investigate the clinical and microbiological characteristics of HLDR *Corynebacterium* infections.

Methods: We first presented a clinical case accompanied by the results of a comprehensive genetic analysis of the isolate, and then performed a systematic scoping review. Based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews, we searched for articles with related keywords, including "*Corynebacterium*", "Daptomycin", and "Resistance", in the MEDLINE and Web of Science databases from the database inception to October 25, 2024. Clinical case reports and research articles documenting the isolation of HLDR *Corynebacterium* species, defined by a minimum inhibitory concentration of DAP at ≥256 μg/mL, were deemed eligible for this review.

Results: Of 80 articles screened, seven case reports detailing eight cases of HLDR *Corynebacterium* infections, as well as five research articles, were included. *C. striatum* was the most common species (7/9 cases, 77.8%), and prosthetic device-associated infections accounted for 66.7% of the cases. Duration of DAP administration before the emergence of HLDR isolates ranged from 5 days to 3 months; three-quarters of the cases developed within 17 days. Three HLDR isolates were genetically confirmed to have an alteration in *pgsA2*. *In vitro* experiments confirmed that *C. striatum* strains acquire the HLDR phenotype at higher rates (71% to 100%) within 24 hours of incubation, compared to other *Corynebacterium* strains

Conclusion: DAP monotherapy, especially for prosthetic device-associated infections, can result in the development of HLDR *Corynebacterium*.

Keywords: antimicrobial resistance, *Corynebacterium*, daptomycin, high-level daptomycin resistance, *pgsA2*.

Introduction

Corynebacterium species are generally considered opportunistic organisms in humans, typically exhibiting susceptibility to antibiotics such as vancomycin and daptomycin (DAP) [1–4]. Recent clinical studies, however, have shown the emergence of high-level daptomycin resistance (HLDR) in *Corynebacterium* species following brief exposure to DAP [5–8]. An *in vitro* study using clinical isolates of DAP-susceptible *Corynebacterium* species (with a breakpoint of ≤ 1.0 μ g/mL) revealed the emergence of HLDR (minimum inhibitory concentration [MIC] value of DAP at ≥ 256 μg/mL) after overnight incubation in a DAP-containing broth [5]. The evolution of DAP nonsusceptibility was experimentally observed in an *in vitro* study following overnight exposure to DAP in 12 of 23 species (*C. afermentans, C. amycolatum, C. aurimucosum, C. bovis, C. jeikeium, C. macginleyi, C. pseudodiphtheriticum, C. resistens, C. simulans, C. striatum, C. tuberculostearicum,* and *C. ulcerans*), which was evident in 50 (31.8%) of 157 isolates examined. This was most pronounced in *C. striatum,* with 32 out of 39 isolates (82.1%) exhibiting the emergence of HLDR [5]. The underlying mechanism of HLDR was attributed to alterations in cell membrane composition, specifically the absence of phosphatidylglycerol (PG) due to mutations leading to the inactivation of phosphatidylglycerol synthase (*pgsA2*) [8–10].

Although increasing cases of HLDR *Corynebacterium* infections have been documented, clinical and microbiological characteristics of such cases are unclear. We recently experienced another clinical case of HLDR *Corynebacterium* infection, which was detected in a patient with *C. striatum*-associated prosthetic valve endocarditis. We herein report this case in detail and the results of a scoping review for clinical cases of HLDR *Corynebacterium* infections and *in vitro* research articles.

Case presentation

A 73-year-old man who had undergone aortic valve replacement surgery for aortic regurgitation 5 years prior visited a previous hospital with a chief complaint of fever. After admission, he was diagnosed with *Enterococcus faecalis* bacteremia and was treated with ampicillin. However, on day 15 of admission, *C. striatum* was detected in his blood culture during ongoing treatment of *E. faecalis* bacteremia. Vancomycin therapy was initiated, but the patient subsequently developed pancytopenia due to vancomycin, necessitating a switch to daptomycin. He was then transferred to our hospital, where he received DAP monotherapy with a favorable clinical course; however, he developed a sudden high fever after 11 days (**Fig. 1**.). Our routine antibiotic susceptibility testing based on the microbroth dilution method (Dry Plate Eiken [Eiken Chemical Co., Ltd, Tokyo, Japan]) suggested that the MIC of DAP increased to the range of non-susceptibility (> 4 μg/mL) (**Supplemental Table 1)**. To confirm the emergence of HLDR, we performed the E-test that corroborated a MIC of DAP increased to >256 μg/mL following DAP exposure (**Fig. 2A, B**).

We then performed polymerase chain reaction (PCR) testing to identify a potential point mutation in *pgsA2* gene, which is associated with reduced PG production. Although the *pgsA2* gene was identified in the HLDR *C. striatum*, the amplified product size was considerably larger than that of the pre-treatment *C. striatum* strain (2,000 bp vs. 600 bp) (**Fig. 2C**). This finding suggested an alteration of *pgsA2* via IS insertion, rather than a point mutation, resulting in the disruption of PG production, based on our previous study [10]. Sequence data of the PCR product was analyzed using the Basic Local Alignment Search Tool (BLAST). The result of the pre-treatment *C. striatum* strain showed a 99.34% concordance with the reference strain of *C. striatum pgsA2* gene (GenBank accession number: LC462282.1), and a 98.95% concordance with the reference strain of *C. striatum* IS30 family transposase gene (GenBank accession number: MZ605120.1) in the HLDR *C. striatum*.

Under the diagnosis of HLDR *C. striatum*-associated prosthetic valve endocarditis, the patient was treated with a combination of teicoplanin and rifampicin and subsequently transferred to another hospital.

Materials and Methods

Study design and strategy

We performed a systematic scoping review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Extension for Scoping Reviews (**Supplemental table 2**) [11,12]. A comprehensive search of MEDLINE and Web of Science databases was performed for all articles published from the database inception to October 25, 2024. We used no filters for the study design and language. The search strategy incorporated pertinent keywords with "*Corynebacterium*" (All Fields), AND "Daptomycin" (All Fields), AND "Resistance" (All Fields).

Eligibility criteria

The inclusion criterion for articles in this review comprised clinical case reports documenting the emergence of HLDR *Corynebacterium* strains during DAP treatment, as well as *in vitro* research articles on HLDR *Corynebacterium* strains. The exclusion criterion was *in vitro* studies or clinical articles on unrelated topics other than the emergence of HLDR *Corynebacterium* strains.

Article selection

Two distinct authors (SF and HA) evaluated selected articles independently, and articles that were deemed appropriate for this study underwent a thorough evaluation. Articles considered eligible were subsequently evaluated in full length. The following data were collected from each case report or research article using a standard data collection form in accordance with PRISMA and Cochrane Collaboration criteria for systematic reviews: year of publication, reporting country, bacterial species, diagnosis, presence of bacteremia and any complications, regimen and duration of DAP therapy, MIC of DAP, alternative antibiotic treatment, genetic investigation for *pgsA2*, and prognosis of the patients.

Results

In the initial search of the MEDLINE and Web of Science databases, 80 articles were detected. Of those, 27 duplicate articles were excluded. In addition, 36 articles were excluded due to being either an *in vitro* investigation or a clinical research article addressing unrelated topics. We found one additional relevant article through other source. The 18 remaining articles underwent further screening, resulting in the exclusion of an additional six articles unrelated to the emergence of HLDR *Corynebacterium* strains during DAP exposure. Finally, seven case reports (eight individual cases) and five *in vitro* research articles were included in our study [5–10,13–18] (**Fig. 3**).

The clinical and microbiological characteristics of nine cases of HLDR *Corynebacterium* infection including our case are summarized in **Table 1**. Although the baseline MIC of DAP was unknown in two cases, the remaining cases showed MIC values of < 0.5 μg/mL based on Etest and broth dilution method before DAP therapy. Progression to HLDR was confirmed by Etest in all strains. *C. striatum* was the most common species (7/9 cases, 77.8%), with one each case of *C. jeikeium* and *C. simulans*. Prosthetic device-associated infections accounted for six cases (66.7%) and bacteremia was reported in eight cases (88.9%). Six patients 66.7%) underwent DAP monotherapy. Although the DAP doses varied among the cases, most of the patients received a sufficient dose (6 mg/kg/day or more). Although the duration of DAP administration before isolating HLDR *Corynebacterium* species ranged from 5 days to 3 months, three-quarters of the cases developed HLDR within 17 days. Vancomycin and linezolid were commonly administered as alternative treatment. A genetic alteration in *pgsA2* was examined in three cases; one case by the splitting of *pgsA2* due to an IS30 insertion [8], another case by a point mutation in *pgsA2* [10], and the last case by IS30 insertion as described above. The prognosis was not reported in three cases but other patients survived, excluding one case caused by *C. jeikeium*.

The characteristics of five *in vitro* research articles on HLDR *Corynebacterium* strains are summarized in **Table 2**. All studies were conducted in the United States, showing an HLDR rate of 71.4–100% in *C. striatum* [5,9,14,17,18] and 11.9–16.1% in other *Corynebacterium* species [5,14]. Genetic analysis was performed in only one research, which the loss-of-function point mutations in PG synthase were detected in HLDR strains [9].

Discussion

We reviewed the clinical and microbiological characteristics of nine clinical cases of HLDR *Corynebacterium* infections that developed during DAP treatment, along with findings from five *in vitro* research. In short, most cases were prosthetic device-associated infections, and HLDR developed within several weeks of DAP therapy in three-quarters of the cases. Regardless of dosage, DAP monotherapy for prosthetic infections appears a risk factor for the development of HLDR in *Corynebacterium* species.

Corynebacterium species potentially cause invasive infections such as bacteremia or infective endocarditis in immunocompromised patients and biofilm-associated infections in patients with prosthetic devices [4,19]. Biofilm formation in

Corynebacterium infections has been reported to be significantly associated with multiple positive blood cultures in a multivariate analysis (odds ratio, 17.4; 95% confidence interval, 3.7–81.9; $p = 0.03$) [20]. *Corynebacterium* species shows 100% susceptibility to vancomycin, teicoplanin, and linezolid, and vancomycin is commonly used for treatment of *Corynebacterium* infections [1–4,18]. *Corynebacterium* species also exhibit high susceptibility to DAP, which is recommended as an alternative therapy when treatment with vancomycin cannot be continued due to renal dysfunction or adverse effects [1–3]. Actually, successful treatment of *Corynebacterium* infections including bacteremia, with DAP has been reported in several cases [21–24]. However, previous studies, including our efforts [8,10], revealed the emergence of HLDR in *Corynebacterium* species shortly after the initiation of DAP treatment.

Development of DAP resistance is a well-known phenomenon in *Staphylococcus aureus*, which is reportedly caused by changes in the composition, metabolism, and permeability of the bacterial cell wall [18]. The increase in MIC of DAP in *S. aureus* is relatively modest, with increases amounting to several-fold, achieving MIC levels of 2–8 µg/mL [25–27]. DAP resistance in *S. aureus* possibly occurs within a span of 2-3 weeks following DAP treatment [28,29], particularly under certain conditions such as monotherapy, suboptimal dosing, disseminated infections, and inadequate drainage or debridement [27,30].

On the contrary, *Corynebacterium* species develop a remarkably higher resistance against DAP, with MIC exceeding 256 µg/mL. *C. striatum* is the most common species that develops the HLDR phenotype, as observed in an *in vitro* study [5]. A previous *in vitro* study demonstrated that DAP loses its bactericidal activity when the membrane PG concentration of the target organism decreases, a phenomenon notably observed in HLDR *C. striatum* compared to other gram-positive bacteria, including *Bacillus subtilis* and *S. aureus*. Although comparative studies on the mechanism of DAP resistance among *Corynebacterium* species with respect to membrane components have not been reported, differences in membrane PG content between *C. striatum* and other *Corynebacterium* species suggested a potential role in resistance mechanism, as indicated in the previous study [5]. The time until the development of HLDR in *Corynebacterium* species ranged from 5 days to 3 months. Notably, seven patients (77.8%) were observed to have HLDR *C. striatum* infections within 17 days. Similarly, *S. aureus* reportedly develops DAP resistance in approximately 3 weeks after initiating DAP treatment [30]. *In vitro* studies showed that *C. striatum* can develop HLDR even after overnight incubation, with incidence rates of 71.4–100% [5,9,14,17,18]. Whereas, progression to HLDR was observed only in 11.9–16.1% of non-striatum *Corynebacterium* species, suggesting the difference in potential of developing the HLDR phenotype among these species. While the actual incidence rate of HLDR *Corynebacterium* strains during DAP treatment remains unknown, clinical reports have documented isolations of DAP non-susceptible *C. striatum* (reported as MIC > 1 µg/mL), indicating that the emergence of HLDR *Corynebacterium* is potentially underestimated [31]. Interestingly, a hetero-resistant population with varying levels of DAP sensitivity has been reported in patients receiving DAP, supporting the hypothesis that such a heterogeneous resistant population may exist and becomes dominant under DAP exposure [5,32]. Given the high frequency observed in *in vitro* studies, it is imperative for clinicians to monitor closely for the rapid emergence of HLDR *Corynebacterium* species even during treatment.

The underlying mechanisms of HLDR *Corynebacterium* species have recently been elucidated. DAP exerts its antibiotic activity by binding to the phospholipid PG in the lipid bilayer of bacterial cell membranes [33]. As a result of genetic mutations in the *pgsA2* gene, which lead to decreased synthesis of PG synthase, DAP shows reduced antimicrobial effectiveness [9]. *pgsA2* is a non-essential gene in some species of Gram-positive bacteria [34], and its genetic alterations therefore do not influence bacterial proliferation. Loss-of-function point mutations or premature stop codon mutations in *pgsA2* are the major mechanisms of HLDR in *Corynebacterium* species [9,10]. We recently found that an IS30 insertion, which results in the splitting of *pgsA2,* can be responsible for the emergence of HLDR in *Corynebacterium* [8]. Although alterations of *pgsA2* were investigated in only three clinical cases, similar genetic changes might have involved the other cases. As for *S. aureus*, DAP resistance is usually associated with dysfunction of lipid biosynthetic enzymes regulated by *mprF* (multiple peptide resistance factor) or *cls* (cardiolipin synthase) [35]. A mutation in *pgsA* has been identified in *S. aureus* and other pathogens as well [36,37]. The difference of HLDR frequency between *C. striatum* and non-striatum *Corynebacterium* may be partially attributed to the mutation susceptibility of the *pgsA2*, warranting further research.

A therapeutic strategy for *Corynebacterium* infections needs to be discussed. In our review, five cases were treated exclusively with DAP monotherapy for prosthetic device-associated infections. To avoid treatment failure due to the emergence of HLDR in *Corynebacterium* strains, antibiotic combination therapy may be advantageous in such cases. Other refractory cases possibly associated with biofilms and a high bacterial load because of disseminated infection would also benefit from the combined treatment. Combinatorial therapies with DAP and rifampicin or linezolid succeeded in treating cases of native-valve endocarditis and thrombophlebitis caused by *Corynebacterium* strains [22,38]. To our knowledge, vancomycin, teicoplanin, and linezolid show 100% sensitivity to *Corynebacterium* species [3,18]. Therefore, we recommend combination therapy with these agents rather than DAP monotherapy for infections associated with prosthetic devices. Further clinical research is warranted to determine the optimal combination regimen.

There are several limitations in our review. First, we did not preregister this study protocol in any registry. Second, the MIC of DAP may not be routinely examined for *Corynebacterium* species at many healthcare facilities. Even if a case of HLDR *Corynebacterium* species was diagnosed, a potential publication bias is unavoidable. Therefore, an underestimation for the clinical significance of HLDR *Corynebacterium* infections is inevitable. Third, we did not evaluate the quality of the selected articles due to no guidelines or guidance on scoping review instructing quality checks for the risk of bias. Despite these concerns, we believe our review of the existing evidence on the clinical cases of HLDR *Corynebacterium* infections will be beneficial to clinicians.

Collectively, we highlighted the clinical and microbiological characteristics of patients infected with HLDR *Corynebacterium* species. Our investigations have revealed that, regardless of dosages, DAP monotherapy for infections associated with prosthetic devices is a significant risk for treatment failure due to the emergence of HLDR *Corynebacterium* strain.

Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable request.

Competing interests

No authors have any competing interests in this case.

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None to report.

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Figure Legends

Figure 1. Clinical and laboratory course of the case.

ABPC, ampicillin; VCM, vancomycin; DAP, daptomycin; TEIC, teicoplanin; RFP, rifampicin; HLDR, high-level daptomycin resistance; MIC, minimum inhibitory concentration.

Blood cultures on day 54 detected HLDR *C. striatum* after 11 days of DAP administration.

Figure 2. *Corynebacterium striatum* isolates before and after DAP treatment.

(A) An E-test result of pre-treatment *C. striatum* isolate showing MIC of DAP at $<$ 0.5 μg/mL

(B) An E-test result of post-DAP treatment *C. striatum* isolate showing MIC of DAP $>$ 256 μg/mL, indicating a high-level daptomycin-resistant phenotype.

(C) Agarose gel electrophoresis of PCR products for *pgsA2*.

Lane M: Size marker. Lane 1: Pre-treatment *C. striatum* isolate. Lane 2: Post-DAP treatment HLDR *C. striatum* isolate. Increased size of the PCR product in *pgsA2* of the HLDR isolate suggests the presence of IS insertion, rather than a point mutation.

DAP, daptomycin; MIC, minimum inhibitory concentration; HLDR, high-level daptomycin resistance.

The E-test was performed on Mueller-Hinton agar plates (Becton Dickinson, Heidelberg, Germany) supplemented with 5% sheep blood at 35°C in a 5% CO_2-enriched atmosphere for 48 hours.

Figure 3. Enrolment flow.