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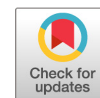

Evaluating the Rapid Emergence of Daptomycin Resistance in *Corynebacterium*: a Multicenter Study

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ABSTRACT Members of the genus *Corynebacterium* are increasingly recognized as pathobionts and can be very resistant to antimicrobial agents. Previous studies have demonstrated that *Corynebacterium striatum* can rapidly develop high-level daptomycin resistance (HLDR) (MIC, ≥256 μg/ml). Here, we conducted a multicenter study to assay for this *in vitro* phenotype in diverse *Corynebacterium* species. *Corynebacterium* clinical isolates ($n = 157$) from four medical centers were evaluated. MIC values to daptomycin, vancomycin, and telavancin were determined before and after overnight exposure to daptomycin to identify isolates able to rapidly develop daptomycin nonsusceptibility. To investigate assay reproducibility, 18 isolates were evaluated at three study sites. In addition, the stability of daptomycin nonsusceptibility was tested using repeated subculture without selective pressure. The impact of different medium brands was also investigated. Daptomycin nonsusceptibility emerged in 12 of 23 species evaluated in this study (*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*) and was detected in 50 of 157 (31.8%) isolates tested. All isolates displayed low (susceptible) MIC values to vancomycin and telavancin before and after daptomycin exposure. Repeated subculture demonstrated that 2 of 9 isolates (22.2%) exhibiting HLDR reverted to a susceptible phenotype. Of 30 isolates tested on three medium brands, 13 (43.3%) had differences in daptomycin MIC values between brands. Multiple *Corynebacterium* species can rapidly develop daptomycin nonsusceptibility, including HLDR, after a short daptomycin exposure period.

KEYWORDS *Corynebacterium*, daptomycin, nonsusceptible, resistance, telavancin, vancomycin

Corynebacterium species have historically been considered normal microbiota of the skin and mucosal surfaces. Increased use of molecular methods that accurately identify *Corynebacterium* to the species level have illuminated species-specific disease associations and helped to demonstrate the potential of *Corynebacterium* for causing invasive infections in immunocompromised patients or biofilm-associated infections in patients with indwelling hardware (1, 2). Some species, including *Corynebacterium striatum*, are noteworthy for multidrug-resistant (MDR) phenotypes. These isolates can be very challenging to treat when they are the causative agent of infection (3–5).

Daptomycin, a lipopeptide antibiotic, is often considered a drug of "last resort" for treatment of Gram-positive bacterial pathogens (6), with demonstrated efficacy against *Staphylococcus* species, *Enterococcus* species, and *Streptococcus* species (7–9). However, as clinical use of daptomycin increases, antimicrobial nonsusceptibility is reported with increasing frequency (10–12).

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In previous reports by our group and others, clinical isolates of *C. striatum* have been observed to rapidly develop high-level daptomycin resistance *in vivo* (HLDR; MIC values of ≥256 μg/ml) (13–16). These findings have been recapitulated *in vitro* with experiments exposing *C. striatum* isolates to daptomycin, in which all isolates evaluated were observed to have the HLDR phenotype (17). The mechanism of HLDR in *C. striatum* has recently been elucidated to occur via loss-of-function mutations in *pgsA2*, encoding an essential enzyme for phosphatidylglycerol (PG) synthesis (18). Mutations in *pgsA2* have also been attributed to HLDR phenotypes in *Staphylococcus aureus*, *Enterococcus faecalis*, and multiple species in the viridans group streptococci (11, 19, 20).

While the findings of prior studies suggest that daptomycin may not be an optimal therapy for infections with *C. striatum*, it remains unclear if other *Corynebacterium* species are as likely to develop daptomycin nonsusceptibility. The objectives of our current multicenter study were to evaluate other clinically relevant isolates in the *Corynebacterium* genus for rapid emergence of daptomycin nonsusceptibility, including HLDR, and to expand the investigation of *C. striatum* clinical isolates to multiple geographic regions in the United States.

MATERIALS AND METHODS

Isolate cohort. Isolates of *Corynebacterium* were collected both prospectively and retrospectively from the following four medical centers: Washington University/Barnes-Jewish Hospital (WU; St. Louis, MO), NorthShore University HealthSystem (NS; Evanston, IL), Weill Cornell Medical Center/NewYork-Presbyterian Hospital (WC; New York City, NY), and Hospital for Special Surgery (HSS; New York City, NY). Isolates were recovered from a variety of clinical specimen types following standard operating procedures at each study site as part of routine patient care. Isolates were included if they were clinically reported to the species level at the study site per the laboratory standard operating procedure, i.e., were deemed clinically relevant in the corresponding culture type. All isolates were sent to WU for characterization. A subset of isolates was later sent to NS and WC for reproducibility testing. Prior to susceptibility testing, each isolate was subcultured two times by the performing laboratory without selective pressure.

Organism identification. Upon arrival at WU, the identification of each study isolate was confirmed using two matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) platforms (MALDI Biotyper [Bruker Daltonics, Inc., Billerica, MA]; Compass Library MBT 8468 MSP and Vitek MS [bioMérieux, Inc., Durham, NC]; Knowledge Base version 3.0). Isolates were assigned a study identifier (see Table S1 in the supplemental material) with the format COR###-A; following overnight exposure to daptomycin, any daptomycin nonsusceptible isolates were archived with the format COR###-B. One isolate, COR158-A, was identified at WC as *C. pyruviciproducens* using 16S rRNA gene sequencing (21).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed using gradient diffusion with Etest for daptomycin and vancomycin, (bioMérieux, Inc.) and MTC MIC test strip for telavancin (Liofilchem, Inc., Waltham, MA). Isolates were tested on Mueller-Hinton agar with 5% sheep blood (MH+B) (Hardy Diagnostics, Santa Maria, CA) and incubated at 35°C in air following Clinical and Laboratory Standards Institute (CLSI) interpretive criteria (22). MIC results were recorded at 24 h and 48 h of incubation, and the 48-h time point data are reported as the MIC. Quality control testing using *Streptococcus pneumoniae* ATCC 49619 and *Staphylococcus aureus* ATCC 29213 was performed each day of testing and assessed using CLSI criteria.

***Corynebacterium*-HLDR assay.** An *in vitro* assay was used to investigate the rapid development of daptomycin nonsusceptibility as previously described (13, 17). Briefly, following organism identification and baseline susceptibility testing, each isolate was inoculated into tryptic soy broth (Becton, Dickinson and Company, Franklin Lakes, NJ) to generate a suspension equivalent to a 0.5 McFarland standard. Two tubes containing 5 ml each were inoculated per isolate, one containing a daptomycin Etest strip cut in half with both halves submerged below the level of the broth as a source of daptomycin for the assay and one as a growth control without the daptomycin Etest strip (Fig. 1). The tubes were incubated overnight at 35°C in air with agitation on a shaker. Following incubation, isolates were tested for susceptibility to daptomycin, vancomycin, and telavancin using gradient diffusion assays.

Reproducibility of the daptomycin resistance phenotype at multiple study sites. A subset of 18 *Corynebacterium* isolates was sent from WU to NS and WC for testing with the *Corynebacterium* HLDR assay described above. Recipients of these isolates were blinded to the organism identification and daptomycin susceptibility status as initially determined at WU.

Stability of the daptomycin resistance phenotype. To investigate the stability of daptomycin nonsusceptibility, nine nonsusceptible isolates (including *C. macginleyi*, *C. ulcerans*, *C. aurimucosum*, *C. simulans*, *C. jeikeium*, *C. amycolatum*, and *C. afermentans*) were serially subcultured on 5% sheep blood agar and incubated at 35°C in air without selective pressure as previously described (13). Briefly, isolates were subcultured from a single colony for 10 consecutive days (or until reversion to daptomycin susceptibility was observed), and daptomycin MIC testing was performed.

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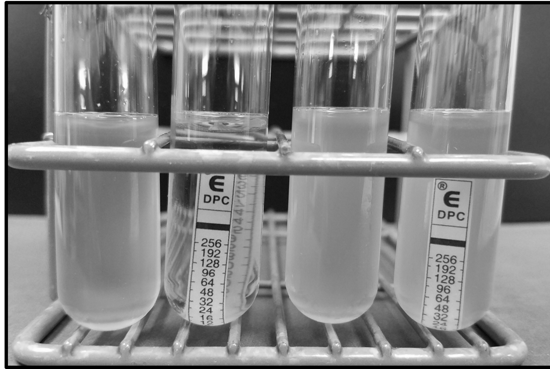

FIG 1 Examples of isolates incubated in broth overnight with and without exposure to daptomycin. Isolate 1 was rated as not visibly turbid following overnight incubation and therefore was deemed susceptible to daptomycin. Isolate 2 was rated as turbid, prompting susceptibility testing of the isolate for daptomycin, vancomycin, and telavancin.

Impact of MH+B medium brand on daptomycin resistance phenotype. To measure the impact of the MH+B medium brand on the *Corynebacterium* HLDR assay described above, we performed testing on 30 isolates (15 *C. striatum* and 15 non-*C. striatum*) using two additional brands of MH+B (Remel [Thermo Fisher Scientific, Lenexa, KS] and BBL [Becton, Dickinson and Company]) in parallel with media from Hardy Diagnostics.

RESULTS

A total of 163 clinical isolates were collected for this study. Of these, 86 originated from WU, 24 from NS, 51 from WC, and 2 from HSS. Five isolates were omitted from the study; two were nonviable for subculture from the freezer stock tubes, two had a final organism identification of *Turicella otitidis*, and one isolate (COR082-A) was a duplicate of another isolate from the same patient. This brought the final sample size to 158 ($n = 39$ *C. striatum*, $n = 119$ non-*C. striatum*). Isolates were derived from a variety of specimen types, with the three most common being respiratory ($n = 39$, including bronchoalveolar lavage fluid, bronchial washing fluid, sputum, tracheal aspirate, pleural fluid, and nasal wounds), blood ($n = 24$), and ventricular assist device driveline wounds ($n = 18$) (Table S1). One isolate of *C. amycolatum* (originating from a driveline wound, isolated at WU), was observed to have HLDR at baseline antimicrobial susceptibility testing; all other isolates were initially susceptible to daptomycin (Fig. 2).

Following subculture and confirmation of organism identification, all isolates were tested for overnight development of daptomycin nonsusceptibility (Table 1, Fig. 2). After daptomycin exposure, 50 of 158 (31.6%) isolates exhibited a nonsusceptible phenotype to daptomycin (susceptible breakpoint, $\leq 1.0\,\mu$g/ml) (Fig. 3B) (22). Nine of these isolates demonstrated a heterogeneous phenotype with a subset of colonies with elevated MIC values (Fig. 3C). The colonies of the subset were reidentified using MALDI-TOF MS to exclude the possibility of a mixed or contaminated culture.

For *C. striatum*, 35 of 39 (89.7%) isolates displayed visible turbidity after overnight growth in broth with daptomycin. Following antimicrobial susceptibility testing of these 35 isolates, 32 displayed HLDR (daptomycin MIC value, $\geq 256\,\mu$g/ml) and 3 remained susceptible to daptomycin (Fig. 2B). Of the 119 non-*C. striatum* isolates, 47 developed visibly turbid cultures after daptomycin exposure, and subsequent testing showed that 18 of the 47 isolates that resulted in turbid cultures were nonsusceptible to daptomycin for an overall nonsusceptibility rate of 15.1% (18/119). MIC values

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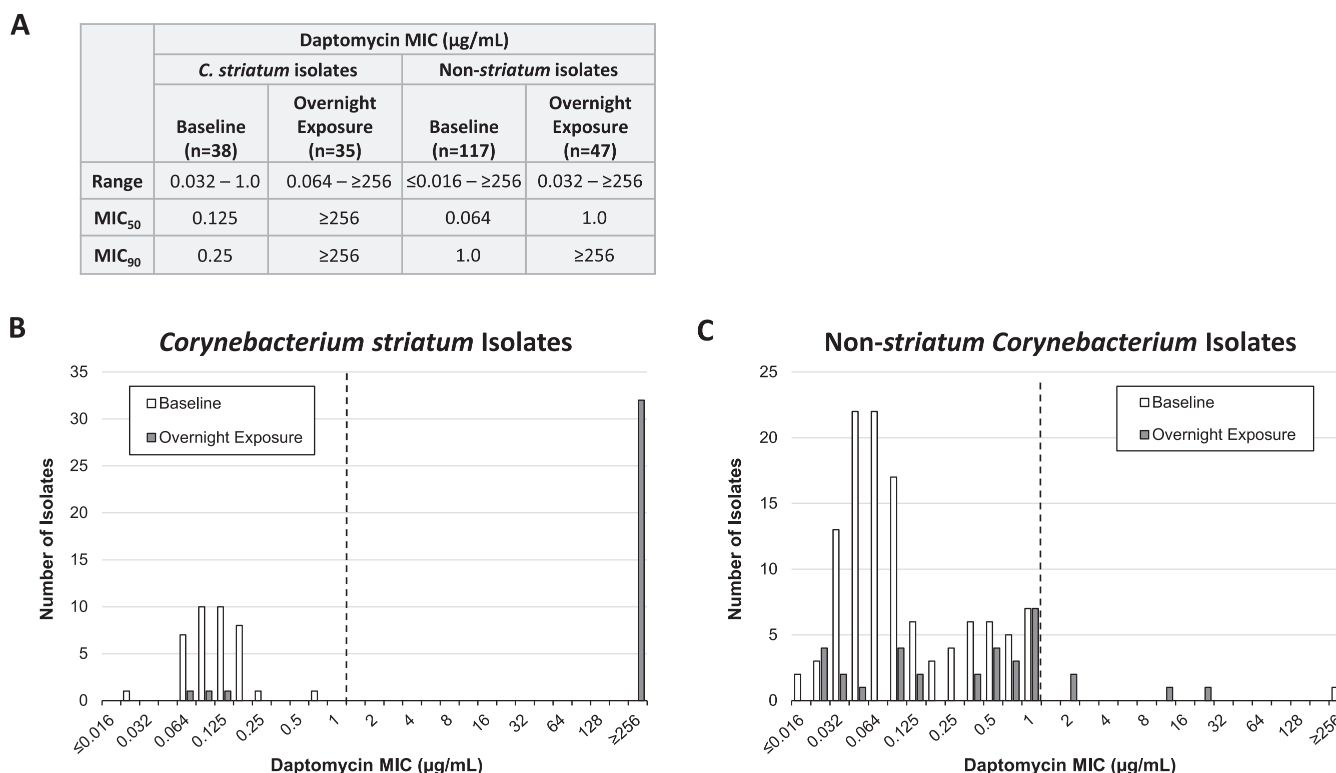

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	Daptomycin MIC (µg/mL)			
	C. striatum isolates		Non-*striatum* isolates	
	Baseline (n=38)	Overnight Exposure (n=35)	Baseline (n=117)	Overnight Exposure (n=47)
Range	0.032 – 1.0	0.064 – ≥256	≤0.016 – ≥256	0.032 – ≥256
MIC_{50}	0.125	≥256	0.064	1.0
MIC_{90}	0.25	≥256	1.0	≥256

FIG 2 Clinical *Corynebacterium* isolates rapidly developed resistance to daptomycin following overnight exposure. (A) Summary table of daptomycin MIC values. (B and C) Bar charts represent the number of isolates for (B) *C. striatum* and (C) non-*C. striatum* isolates with daptomycin MIC values before and after overnight daptomycin exposure. The dashed line indicates the susceptible breakpoint for daptomycin (≤1.0 µg/ml is susceptible).

following overnight exposure ranged from 2.0 to ≥256 µg/ml, with 14 of these being HLDR (Fig. 2C).

All isolates were also assayed for MICs to vancomycin and telavancin (before and after overnight daptomycin exposure. All 158 isolates displayed low vancomycin (susceptible) and telavancin MIC values at baseline and maintained these low MIC values after daptomycin exposure (Fig. 4). Of note, there are no established breakpoints available for telavancin with *Corynebacterium* species; however, the highest MIC value observed was 0.25 µg/ml before daptomycin exposure, with a range of 0.016 to 0.125 µg/ml after daptomycin exposure, implying telavancin susceptibility before and after daptomycin exposure.

To investigate the stability of the daptomycin-nonsusceptible phenotype in non-*C. striatum* species, nine isolates that had been found to develop daptomycin nonsusceptibility were randomly selected and subcultured 10 times without selective pressure (Table 2). Two isolates were found to lose the HLDR phenotype after repeated passaging; COR007-B (*C. macginleyi*) had an initial daptomycin MIC value of ≥256 µg/ml and had an MIC value of 0.064 µg/ml after seven passages; COR069-B (*C. jeikeium*) also started with a daptomycin MIC value of ≥256 µg/ml and had an MIC value of 1.0 µg/ml after five passages (Fig. 5). Of note, COR069-A (the parent strain for COR069-B) had expressed a resistant subpopulation during initial testing: the majority of the growth had a daptomycin MIC value of 1.0 µg/ml, but a resistant subpopulation of colonies had an MIC value of ≥256 µg/ml.

To investigate the reproducibility of our HLDR findings, 18 isolates were tested at WU, NS, and WC for the development of daptomycin nonsusceptibility. Initially 12 (66.7%) yielded the same phenotype at all three sites (Table 3; trial 1). As part of the investigation for discrepant results, we hypothesized that each study site may have regarded the growth in broth differently, i.e., evaluation of turbidity and the decision to perform susceptibility testing after daptomycin exposure. To address this, repeat

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TABLE 1 Clinical isolates of *Corynebacterium* and their daptomycin susceptibility status before and after *in vitro* exposure to daptomycin

***Corynebacterium* sp.**	**No. of isolates by study site [*n* (WU, NS, WC, HSS)] (*n* = 157)**	**No. of daptomycin-nonsusceptible isolates at baseline (*n* = 1)**	**No. of daptomycin-nonsusceptible isolates after overnight exposure (*n* = 50)**
C. accolens	1 (0, 0, 1, 0)	0	0
C. afermentans	3 (3, 0, 0, 0)	0	1[a] (1)
C. amycolatum	42 (31, 3, 8, 0)	1	1
C. aurimucosum	3 (1, 2, 0, 0)	0	2
C. bovis	3 (0, 0, 3, 0)	0	1[a] (1)
C. coyleae	1 (1, 0, 0, 0)	0	0
C. diphtheriae	2 (2, 0, 0, 0)	0	0
C. imitans	1 (1, 0, 0, 0)	0	0
C. jeikeium	13 (9, 0, 3, 1)	0	4[a] (4)
C. kroppenstedtii	3 (2, 0, 1, 0)	0	0
Corynebacterium lipophile group	1 (0, 1, 0, 0)	0	0
C. lipophiloflavum	1 (0, 1, 0, 0)	0	0
C. macginleyi	6 (5, 0, 1, 0)	0	4[a] (1)
C. mucifaciens	1 (1, 0, 0, 0)	0	0
C. propinquum	7 (0, 3, 4, 0)	0	0
C. pseudodiphtheriticum	12 (4, 2, 6, 0)	0	1
C. pyruviciproducens	1 (0, 0, 1, 0)	0	0
C. resistens	1 (0, 0, 1, 0)	0	1
C. simulans	1 (0, 1, 0, 0)	0	1
C. striatum	39 (8, 10, 20, 1)	0	32[a] (1)
C. tuberculostearicum	9 (9, 0, 0, 0)	0	1[a] (1)
C. ulcerans	3 (3, 0, 0, 0)	0	1
C. urealyticum	3 (2, 1, 0, 0)	0	0

[a]One or more isolates exhibited a nonsusceptible subpopulation; the values in parentheses indicate the number of isolates with this phenotype. WU, Washington University/Barnes Jewish Hospital; NS, NorthShore University HealthSystem; WC, Weill Cornell Medical Center/NewYork-Presbyterian Hospital; HSS, Hospital for Special Surgery.

testing was performed and included follow-up susceptibility testing regardless of an isolate's turbidity in broth (Table 3; trial 2). This yielded congruent results between all three study sites for 15 of the 18 (83.3%) isolates.

We further hypothesized that differences in medium brand for MH+B agar may have accounted for differences in observed phenotype; WU used Hardy media, while NS and WC both used BBL. To investigate the possible impact of medium brand on the expression of a rapidly developing daptomycin-nonsusceptible phenotype, 30 isolates (15 *C. striatum*, 15 non-*C. striatum*) were tested at WU on all three types of commercially available MH+B agar (Hardy, BBL, and Remel). This evaluation included the 6 isolates that were initially found to be discrepant during reproducibility testing at the three study sites. Of the 30 isolates, 13 (43.3%) were found to have some difference in their daptomycin susceptibility phenotype between the three medium brands (2 *C. striatum* and 11 non-*C. striatum*) (Tables 4 and 5). These differences involved either a frank change from susceptible to nonsusceptible status or a situation in which a nonsusceptible subpopulation of colonies in the zone of growth inhibition was observed only on certain medium brands (Fig. 6). Of note, the three isolates with discrepant results between study sites during reproducibility testing (COR069-A, COR109-A, and COR147-A) each retained their phenotypes when tested on the three medium brands.

DISCUSSION

Corynebacterium bacteria have traditionally been regarded as largely commensal organisms, but members of this genus are pathogens and pathobionts. Some species are associated with distinct clinical presentations (e.g., *C. macginleyi* and ocular infections), while others, including *C. striatum*, are associated with device-associated infections and MDR (1–4). Daptomycin, a lipopeptide antibiotic, initially showed great promise as a therapeutic option for *C. striatum* infections. However, studies from our group and others demonstrated the rapid emergence of daptomycin resistance in

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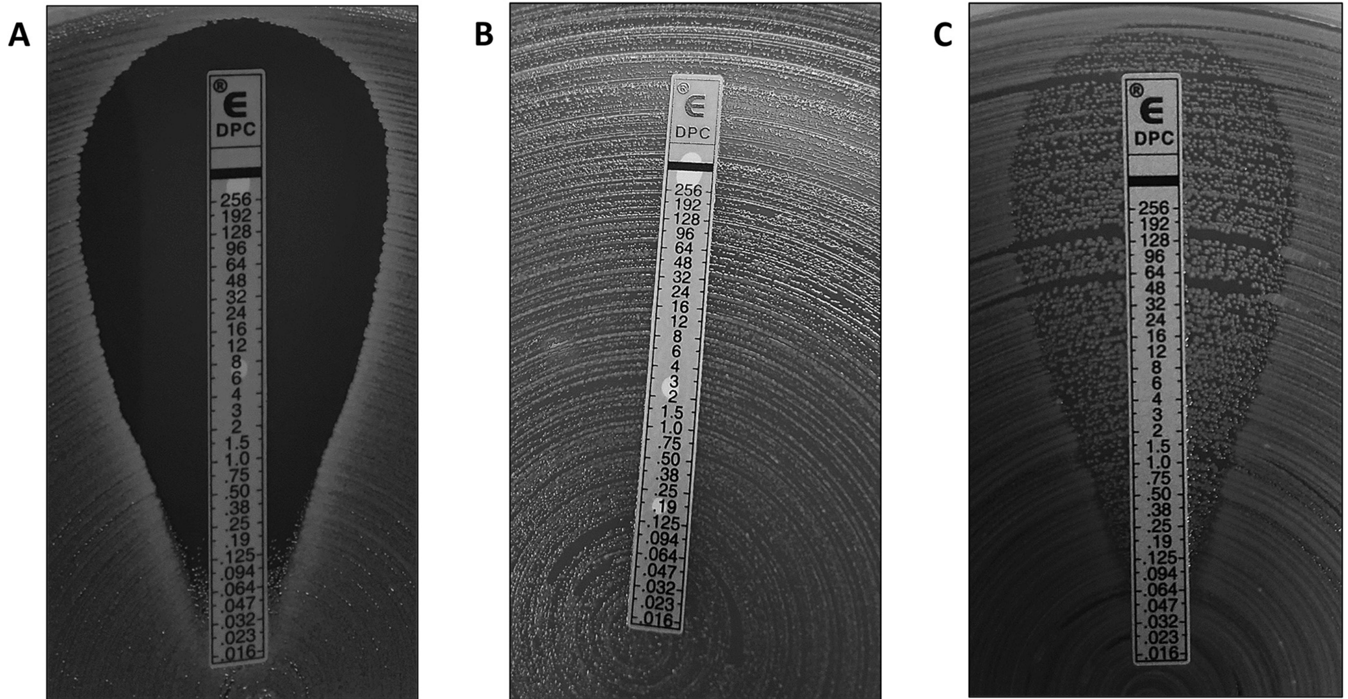

FIG 3 (A to C) Examples of non-*C. striatum* isolates with (A) uniform susceptibility (MIC, 0.25 μg/ml), (B) uniform nonsusceptibility (MIC, ≥256 μg/ml), and (C) a nonsusceptible subpopulation of colonies to daptomycin (MIC, ≥256 μg/ml). Plates represent MIC testing of isolates following their overnight exposure to daptomycin.

this species (13, 14, 16, 17). While there are limited data available for non-*C. striatum* isolates, two case reports described daptomycin-resistant isolates of *C. jeikeium* and *C. propinquum* from a bloodstream infection and a case of prosthetic valve endocarditis, respectively (23, 24). In both cases, the patients had been treated with daptomycin prior to antimicrobial susceptibility testing. Here, we aimed to expand upon these investigations by testing a diverse group of clinically relevant *Corynebacterium* species isolates as well as additional *C. striatum* clinical isolates from multiple geographic sites.

After exposing 158 clinical *Corynebacterium* isolates to daptomycin overnight, we observed the rapid development of daptomycin resistance in 50 (31.6%) isolates (32 *C. striatum* and 18 non-*C. striatum*). The majority of these developed the phenotype previously referred to as HLDR, i.e., isolates with a daptomycin MIC value of ≥256 μg/ml (18). However, among 4 non-*C. striatum* isolates, we observed a MIC value that was lower but still above the CLSI breakpoint for susceptibility to daptomycin. In addition, 9 isolates (1 *C. striatum* and 8 non-*C. striatum*) expressed a subset of colonies with HLDR (which was officially interpreted as a MIC of ≥256 μg/ml), but the majority of the population was susceptible to daptomycin (Fig. 3C). Further, the repeated subculture of several isolates (including one that had initially expressed a resistant subpopulation) without selective pressure indicated a potential for complete reversal of HLDR among certain non-*C. striatum* isolates. Of note, one isolate of *C. amycolatum* (COR081-A) from a driveline wound had a baseline MIC value of ≥256 μg/ml; medical chart review revealed that this patient had been treated for 6 weeks with daptomycin.

Collectively, these results differ from our group's previous observations of uniform, stable HLDR among *C. striatum* isolates *in vitro* as evaluated with standardized antimicrobial susceptibility testing (13, 17). Interestingly, during our prior trials of the *Corynebacterium*-HLDR assay with *C. striatum*, a lag was observed during growth in broth with daptomycin at 4 h of incubation compared to controls (although no lag was discernible by 24 h) (13). We hypothesized that this could be due to the

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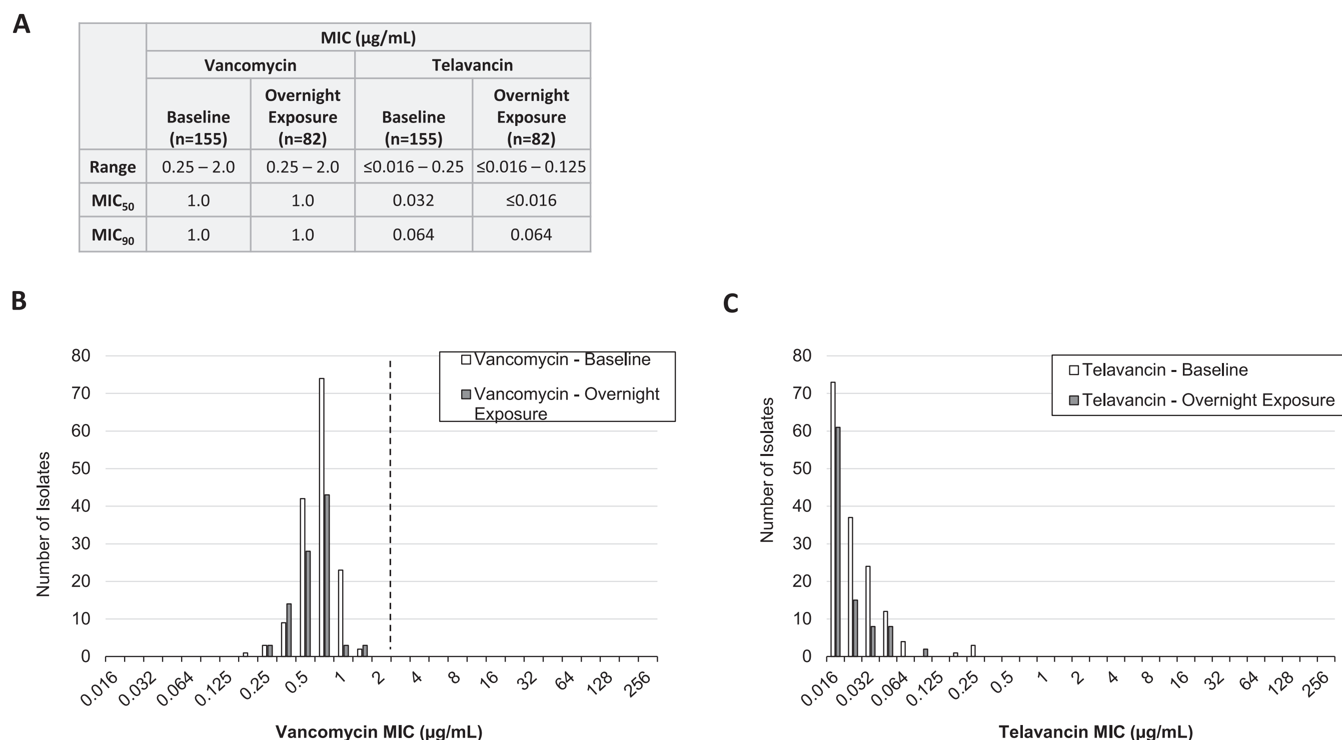

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	MIC (µg/mL)			
	Vancomycin		Telavancin	
	Baseline (n=155)	Overnight Exposure (n=82)	Baseline (n=155)	Overnight Exposure (n=82)
Range	0.25 – 2.0	0.25 – 2.0	≤0.016 – 0.25	≤0.016 – 0.125
MIC_{50}	1.0	1.0	0.032	≤0.016
MIC_{90}	1.0	1.0	0.064	0.064

FIG 4 All *Corynebacterium* isolates exhibited low MIC values to vancomycin (susceptible) and telavancin (indicative of susceptibility) before and after daptomycin exposure. (A) Summary table of vancomycin and telavancin MIC values. (A and B) Bar charts represent the number of isolates tested for (B) vancomycin and (C) telavancin before and after overnight daptomycin exposure. The dashed line indicates the susceptible breakpoint for vancomycin (MIC, ≤8.0 µg/ml). There are no telavancin breakpoints available for *Corynebacterium* species.

presence of a heteroresistant population, which was selected in the presence of daptomycin. In a study by another group, a clinical isolate of *C. striatum* originating from a case of native valve endocarditis was reported to exhibit two subpopulations upon testing with daptomycin—one with a MIC value of ≥256 µg/ml and one with a MIC value of 0.094 µg/ml (15). Together with our current study, these findings suggest that, for at least some isolates, both *C. striatum* and other *Corynebacterium* species may demonstrate a mixed resistance phenotype following exposure to daptomycin.

The structural similarities between daptomycin (a lipopeptide) and telavancin (a lipoglycopeptide) prompted us to investigate the impact of daptomycin exposure to telavancin as well as vancomycin (a glycopeptide). Our entire isolate cohort was susceptible to both vancomycin and telavancin, both before and after exposure to daptomycin. These results are congruent with our previous large study of *C. striatum* iso-

TABLE 2 Impact of repeated subculture without selective pressure on the daptomycin-nonsusceptible phenotype

Study identifier	*Corynebacterium* sp.	Daptomycin MIC prior to subculture (µg/ml)	Daptomycin MIC after 10 subcultures (µg/ml)
COR007-B[a]	*C. macginleyi*	≥256	0.064
COR013-B	*C. macginleyi*	≥256	≥256
COR016-B	*C. ulcerans*	≥256	≥256
COR057-B	*C. aurimucosum*	16.0	16.0
COR060-B	*C. simulans*	≥256	≥256
COR069-B[a]	*C. jeikeium*	≥256	1.0
COR081-B	*C. amycolatum*	≥256	≥256
COR103-B	*C. aurimucosum*	≥256	≥256
COR109-B	*C. afermentans*	2.0	2.0

[a]Isolates COR007-B and COR069-B were subcultured for 7 and 5 days, respectively.

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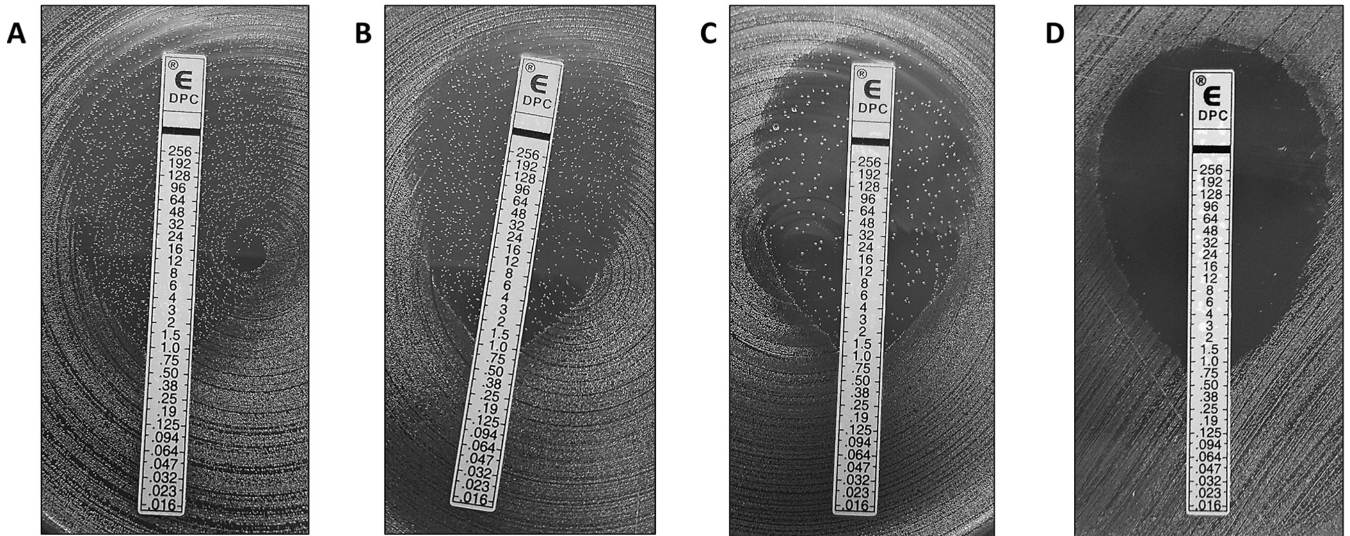

FIG 5 Instability of the daptomycin nonsusceptibility phenotype in an isolate of *C. macginleyi* (COR069-B). Following repeated subculture without selective pressure, the MIC values to daptomycin were tested. (A to D) Representative images are shown for subcultures 1 (A), 2 (B), 3 (C), and 4 (D). Additional subculturing was performed for days 5 to 10 but yielded a single population with an MIC value of 1.0 μg/ml, i.e., susceptible (no resistant subpopulation was noted).

lates (17). The notion that exposure to one of these agents can potentially lead to resistance to a second drug has been demonstrated using *S. aureus*, in which vancomycin induces resistance to daptomycin, presumably through cell wall thickening (25–28). In many studies, isolates were derived from daptomycin-naive patients. The impact of vancomycin exposure on daptomycin susceptibility represents an interesting area of future study with *Corynebacterium*.

The *Corynebacterium* HLDR assay conducted throughout this study represents a potential workflow for clinical laboratories to assess for rapid development of daptomycin resistance in *Corynebacterium* isolates. Thus, a large portion of our study aimed

TABLE 3 Reproducibility of *Corynebacterium* HLDR testing at three study sites[a]

		Data for WU (Hardy MH+B) (μg/ml)				Data for NS (BBL MH+B) (μg/ml)				Data for WC (BBL MH+B) (μg/ml)					
		Trial 1		Trial 2		Trial 1		Trial 2		Trial 1		Trial 2			
Study identifier	*Corynebacterium* sp.	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Trial 1: sites in agreement	Trial 2: sites in agreement
COR019-A	*C. striatum*	0.064	≥256	0.125	≥256	0.125	NA	0.125	≥256	0.064	≥256	0.064	≥256	2	3
COR025-A	*C. striatum*	0.064	≥256			0.125	≥256			0.064	≥256			3	3
COR032-A	*C. propinquum*	0.032	0.032			0.064	0.032			0.064	NA			3	3
COR033-A	*C. amycolatum*	0.064	NA			0.064	NA			0.032	NA			3	3
COR036-A	*C. pseudodiphtheriticum*	0.064	NA			0.125	NA			0.125	NA			3	3
COR057-A	*C. aurimucosum*	0.032	32.0			0.032	8.0			0.032	8.0			3	3
COR060-A	*C. simulans*	0.125	≥256	0.25	≥256	0.125	NA	0.125	≥256	0.25	NA	0.125	≥256	2	3
COR062-A	*C. urealyticum*	0.125	0.5			0.125	0.064			0.125	0.125			3	3
COR069-A	*C. jeikeium*	0.50	≥256	0.50	≥256	0.25	0.032	0.5	0.25	0.50	0.25	0.25	NA	2	2
COR071-A	*C. jeikeium*	1.0	1.0			1.0	4.0			1.0	1.0			3	3
COR081-A	*C. amycolatum*	≥256	≥256			≥256	≥256			192	≥256			3	3
COR103-A	*C. aurimucosum*	≤0.016	16.0	0.032	≥256	0.032	8.0	0.032	8.0	<0.016	NA	≤0.016	16.0	2	3
COR109-A	*C. afermentans*	0.5	≥256	0.25	≥256	0.125	2.0	0.25	≥256	0.25	NA	0.25	NA	2	2
COR111-A	*C. amycolatum*	0.064	NA			0.125	NA			0.125	NA			3	3
COR122-A	*C. pseudodiphtheriticum*	0.032	NA			0.0625	NA			0.032	NA			3	3
COR129-A	*C. striatum*	0.25	≥256			0.125	≥256			0.125	≥256			3	3
COR147-A	*C. striatum*	0.125	0.064	0.064	0.064	0.125	≥256	0.125	≥256	0.125	≥256	0.125	≥256	2	2
COR151-A	*C. kroppenstedtii*	0.50	NA			0.25	NA			0.50	NA			3	3

[a] "Pre" and "Post" refer to daptomycin MIC values (μg/ml) at baseline and after overnight exposure to daptomycin, respectively. For isolates marked "NA," no turbidity was observed following growth in broth with daptomycin; therefore, postexposure testing was not performed. WU, Washington University/Barnes Jewish Hospital; NS, NorthShore University HealthSystem; WC, Weill Cornell Medical Center/NewYork-Presbyterian Hospital; HSS, Hospital for Special Surgery; BBL, Becton, Dickinson and Company.

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TABLE 4 Testing for overnight daptomycin nonsusceptibility with three different medium brands of MH+B agar[a]

		BBL		Hardy		Remel		No. of medium types with phenotype agreement
Study ID	*Corynebacterium* sp.	Pre	Post	Pre	Post	Pre	Post	
COR006-A	*C. tuberculostearicum*	0.125	≥256 (0.125)	0.25	≥256 (0.25)	0.032	≥256 (0.125)	3
COR007-A	*C. macginleyi*	0.125	0.064	0.125	≥256	0.125	≥256 (0.064)	0
COR013-A	*C. macginleyi*	0.125	≥256 (0.032)	0.125	≥256 (0.064)	0.064	≥256 (0.032)	3
COR016-A	*C. ulcerans*	0.125	≥256 (0.125)	0.125	4 (0.064)	0.125	32 (0.032)	3
COR019-A	*C. striatum*	0.125	≥256	0.125	≥256	0.125	≥256	3
COR023-A	*C. jeikeium*	0.25	8	0.25	2	0.125	0.5	2
COR024-A	*C. ulcerans*	0.25	0.25	0.5	2	0.50	0.5	2
COR025-A	*C. striatum*	0.125	≥256	0.25	≥256	0.064	≥256	3
COR030-A	*C. striatum*	0.064	≥256	0.125	≥256	0.032	≥256	3
COR031-A	*C. striatum*	0.032	≥256	0.125	≥256	0.125	≥256	3
COR037-A	*C. striatum*	0.125	≥256	0.125	≥256	0.125	≥256	3
COR040-A	*C. bovis*	0.25	0.5	0.25	2	0.125	0.5	2
COR044-A	*C. striatum*	0.125	≥256 (4)	0.125	≥256 (4)	0.064	≥256 (4)	3
COR046-A	*C. striatum*	0.125	≥256	0.032	≥256	0.032	≥256	3
COR049-A	*C. striatum*	0.25	≥256	0.25	≥256	0.125	≥256	3
COR057-A	*C. aurimucosum*	≤0.016	128 (32)	0.064	128	0.032	≥256 (16)	2
COR060-A	*C. simulans*	0.25	≥256 (0.064)	0.25	≥256	0.125	≥256	2
COR063-A	*C. jeikeium*	0.25	0.25	0.032	≥256 (0.064)	0.25	0.032	2
COR067-A	*C. jeikeium*	0.50	1	0.5	6	0.25	1	2
COR069-A	*C. jeikeium*	0.50	0.25	0.50	≥256	0.25	≥256	2
COR081-A	*C. amycolatum*	64	≥256	≥256	≥256	≥256	≥256	3
COR103-A	*C. aurimucosum*	≤0.016	32	0.032	≥256	≤0.016	≥256 (64)	0
COR109-A	*C. afermentans*	0.25	≥256 (0.25)	0.25	≥256	0.25	≥256 (0.125)	2
COR128-A	*C. striatum*	0.032	≥256	0.50	≥256	0.25	≥256	3
COR129-A	*C. striatum*	0.25	0.125	0.25	0.125	0.25	0.25	3
COR134-A	*C. striatum*	0.125	≥256	0.125	≥256	0.064	≥256	3
COR138-A	*C. striatum*	0.125	≥256	0.125	≥256	0.064	≥256	3
COR141-A	*C. striatum*	0.25	≥256	0.125	≥256 (0.5)	0.25	≥256	2
COR147-A	*C. striatum*	0.032	≥256	0.064	0.064	0.064	≤0.016	2
COR163-A	*C. striatum*	0.125	≥256	0.125	≥256	0.25	≥256	3

[a]"Pre" and "Post" refer to daptomycin MIC values (μg/ml) at baseline and after overnight exposure to daptomycin, respectively. Values in parentheses indicate the MIC values observed for a subpopulation of colonies. Phenotype agreement refers to the susceptibility interpretation and/or observation of a nonsusceptible subpopulation, if applicable.

to investigate how prevalent daptomycin nonsusceptibility was in a diverse group of *Corynebacterium* clinical isolates and the reproducibility of results when this assay was performed at different study sites. Initially, our results were only 66% in agreement, prompting discussion among the three sites about the details of experimentation. We realized that variability may have been introduced due to the subjective nature of assessing the turbidity of isolates grown in broth with daptomycin; i.e., one site may have set up post-daptomycin exposure MIC testing, while others did not. This could be especially difficult to gauge for lipophilic non-*C. striatum Corynebacterium* species, as the isolates evaluated in the experiments did not always have robust growth, even in broth control tubes. When repeat testing was performed for discrepant isolates in which all broths were set up post-daptomycin exposure, results were 83.3% in agreement. This discrepancy could be mitigated in a laboratory workflow by requiring post-

TABLE 5 Testing for overnight daptomycin nonsusceptibility with three different medium brands of MH+B agar[a]

	BBL		Hardy		Remel	
MIC summary statistics	Pre	Post	Pre	Post	Pre	Post
Median	0.125	≥256	0.125	≥256	0.125	≥256
Mode	0.125	≥256	0.125	≥256	0.125	≥256
Minimum	≤0.016	0.064	0.032	0.064	≤0.016	≤0.016
Maximum	64	≥256	≥256	≥256	≥256	≥256

[a]"Pre" and "Post" refer to daptomycin MIC values (μg/ml) at baseline and after overnight exposure to daptomycin, respectively.

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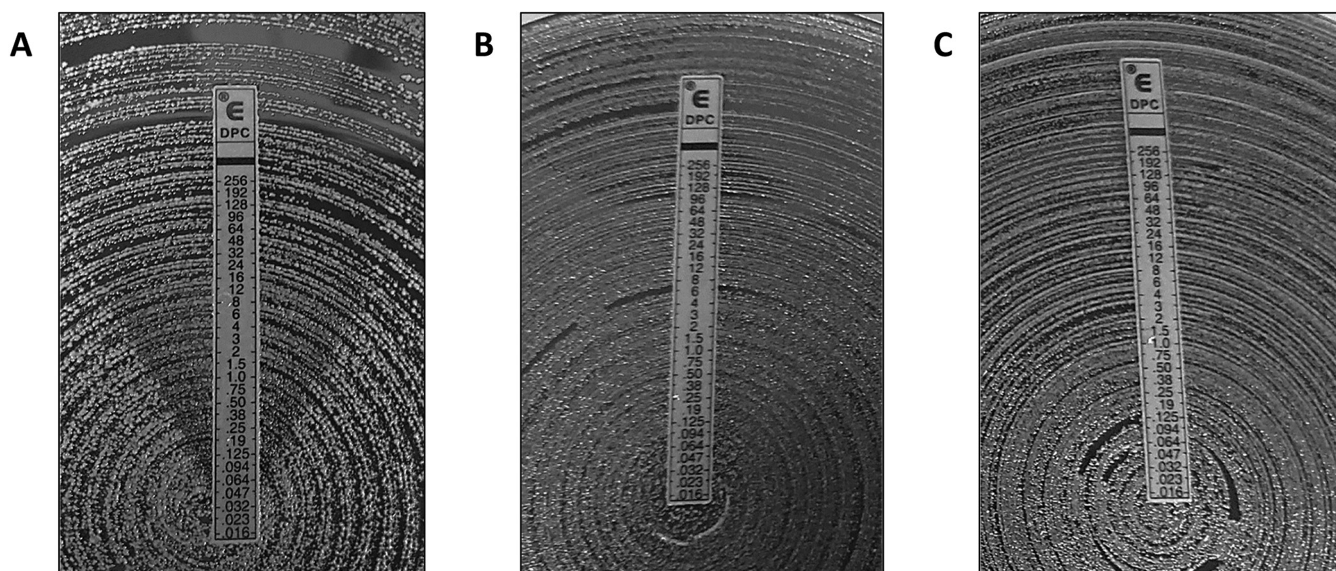

FIG 6 Example of daptomycin-nonsusceptible phenotype variability during testing on three commercially available brands of Mueller-Hinton with blood agar. (A to C) An isolate of *C. simulans* (COR060-A) was exposed to daptomycin in broth overnight and then tested for daptomycin MIC values on the following three different medium brands: (A) BBL, (B) Hardy, and (C) Remel. A resistant subpopulation of colonies was only observed when tested on BBL medium.

daptomycin exposure testing for non-*C. striatum Corynebacterium* isolates, regardless of visible turbidity.

The discussion among study sites also revealed that two different brands of MH+B had been utilized for antimicrobial susceptibility testing, leading us to test three commercially available brands of MH+B media in parallel at a single site. When a subset of isolates was tested at a single site on the different medium brands, 43.4% of isolates gave different results. In addition, the remaining three discrepant results from intersite reproducibility testing were explained by the observed phenotype differences of the three medium brands. There was no clear trend between medium type and observed daptomycin nonsusceptibility phenotype, including the expression of nonsusceptibility. While the major components of MHB agar are the same between the three medium brands, our findings could be explained by subtle differences in medium lipid composition, blood source, or other manufacturing differences (29).

We observed an unstable daptomycin-nonsusceptible phenotype for two non-*C. striatum* isolates, which differs from our previous findings with *C. striatum*. This raises the possibility that there could be differences in the mechanism(s) leading to daptomycin resistance between *C. striatum* and non-*C. striatum* species. An interesting area of future study could employ lipidomics to investigate differences in the membrane phosphatidylglycerol (PG) composition of non-*C. striatum Corynebacterium* species; previous studies from our group used this approach to demonstrate that PG content is greatly reduced in HLDR strains of *C. striatum* (18). Characterization of PG content in other species could help explain the differences in phenotypes we observed between the majority of *C. striatum* and non-*C. striatum* species. Also, subtle differences in the number of subcultures from frozen stocks may have impacted the observed differences in phenotypes between study sites; each isolate was subcultured an additional time at WU prior to being sent to the other two study sites.

Our study has several limitations, including evaluation of isolates that were initially recovered from the respiratory tract (39 of 155, or 25.2%). We acknowledge that daptomycin is not used in the treatment of pneumonia; however, these isolates were included in our study, as they represent important causes of infection and have the

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potential for progression to disseminated disease beyond the respiratory tract. An additional study limitation is posed by the rarity of certain species (several species had only a single representative isolate); therefore, our findings from these species may not translate to other isolates of these species.

The strengths of our study are the inclusion of many *Corynebacterium* clinical isolates collected from multiple study sites over several years, representing many clinically relevant species. An additional strength is the inclusion of isolates collected both retrospectively and prospectively.

Together, our findings suggest that multiple *Corynebacterium* species, in addition to *C. striatum*, are able to rapidly develop resistance to daptomycin. This has important clinical implications, especially for conditions where daptomycin may be used for long-term treatment of *Corynebacterium* infections.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.4 MB.

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