

Addition of Ceftaroline to Daptomycin after Emergence of Daptomycin-Nonsusceptible *Staphylococcus aureus* during Therapy Improves Antibacterial Activity

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Antistaphylococcal beta-lactams enhance daptomycin activity and have been used successfully in combination for refractory methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Ceftaroline possesses MRSA activity, but it is unknown if it improves the daptomycin potency comparably to other beta-lactams. We report a complex patient case of endocarditis who was treated with daptomycin in combination with ceftaroline, which resulted in clearance of a daptomycin-nonsusceptible strain. An *in vitro* pharmacokinetic/pharmacodynamic model of renal failure was used to simulate the development of daptomycin resistance and evaluate the microbiologic effects of daptomycin plus ceftaroline treatment. Combination therapy with daptomycin and ceftaroline restored daptomycin sensitivity *in vivo* and resulted in clearance of persistent blood cultures. Daptomycin susceptibility *in vitro* was increased in the presence of either ceftaroline or oxacillin. Daptomycin at 6 mg/kg of body weight every 48 h was bactericidal in the model but resulted in regrowth and daptomycin resistance (MIC, 2 to 4 $\mu\text{g/ml}$) with continued monotherapy. The addition of ceftaroline at 200 mg every 12 h after the emergence of daptomycin resistance enhanced bacterial killing. Importantly, daptomycin plus ceftaroline as the initial combination therapy produced rapid and sustained bactericidal activity and prevented daptomycin resistance. Both *in vivo*- and *in vitro*-derived daptomycin resistance resulted in bacteria with more fluid cell membranes. After ceftaroline was added in the model, fluidity was restored to the level of the initial *in vivo* isolate. Daptomycin-resistant isolates required high daptomycin exposures (at least 10 mg/kg) to optimize cell membrane damage with daptomycin alone. Ceftaroline combined with daptomycin was effective in eliminating daptomycin-resistant MRSA, and these results further justify the potential use of daptomycin plus beta-lactam therapy for these refractory infections.

Daptomycin is increasingly used in the treatment of complex methicillin-resistant *Staphylococcus aureus* (MRSA) infections. Reduced susceptibility to daptomycin in *S. aureus* (MIC, $>1 \mu\text{g/ml}$) has been attributed to a number of genetic mutations, most notably in the *mprF* locus, that correlate with a phenotype characterized by altered cell walls, surface charge, and membrane function (18, 21). These characteristics have often been associated with concomitant vancomycin-intermediate susceptibility in *S. aureus* (VISA), with vancomycin MICs of $\geq 4 \mu\text{g/ml}$, but this cross-resistance is not absolute (22).

A number of recent studies have described novel interactions between beta-lactam antibiotics and daptomycin in MRSA strains (7, 11, 31). As first described by Yang et al., reduced daptomycin susceptibility correlates with a simultaneous increase in oxacillin susceptibility, even though the beta-lactam resistance element *mecA* remains intact (31). *In vitro* and *in vivo* static and dynamic assessments of daptomycin in combination with antistaphylococcal beta-lactams demonstrate rapid antimicrobial synergy by enhanced daptomycin binding to the cell membrane (7, 26). Via a similar mechanism, beta-lactam antibiotics enhance the killing activities of cationic host defense peptides (26). The potential therapeutic implications for this combination are compelling, especially in cases with a high risk of treatment failure and when treatment options are limited.

We present a recent case of a woman admitted to the intensive care unit from an outside hospital who had MRSA bacteremia and septic shock. The patient's past medical history was significant for

end-stage renal disease with thrice-weekly hemodialysis, diabetes mellitus type II, and morbid obesity. The patient had failed 11 total days of daptomycin treatment at 6 mg/kg of body weight every 48 h at the outside hospital, as well as in our hospital, and had 12 consecutive total positive blood cultures. Due to access issues, intravenous catheters could not be removed. As shown in the antibiotic timeline in Fig. 1, the failing daptomycin therapy was supplemented by the addition of ceftaroline after the development of reduced daptomycin susceptibility, persistent positive cultures, and clinical sepsis. A transesophageal echocardiogram (TEE) on hospital day 11 demonstrated a 23-mm mass in the right atrium. On hospital day 18, the patient complained of right wrist pain; subsequent aspiration of this tissue revealed MRSA. A repeat TEE on hospital day 63 (day 54 of combination therapy) showed the atrial mass was smaller (11 mm by 10 mm) and no longer mobile. Combination therapy was followed by clearance of blood cultures within 4 days and slow improvement in symptoms, but

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Hospital Day	Presentation from outside hospital →																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Daptomycin ^a	6 ^b		6		6		6		6		6		6		6		6		10 ^c	10
Ceftaroline 200 mg every 12 h																				
MRSA blood culture	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Renal replacement method			iHD				iHD					iHD		iHD		iHD				iHD

^aDaptomycin administered after hemodialysis session on days with concomitant intermittent hemodialysis (iHD)
^b6 mg/kg dose
^c10 mg/kg dose

FIG 1 Course of therapy timeline until definitive clearance of bacteremia in a patient with prosthetic valve endocarditis treated with daptomycin alone and then combined with ceftaroline. Following the collection of the timeline data presented here, the patient was hospitalized for an additional 48 days, received combination therapy throughout, and all remaining blood cultures were negative.

other medical complications resulted in hospital discharge to home hospice.

In this study, we further investigate the microbiologic effects of the antimicrobial treatment noted in this case by utilizing an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model of daptomycin alone and in combination with ceftaroline to (i) treat a daptomycin-nonsusceptible strain that develops while on therapy and (ii) attempt to prevent the emergence of this phenotype with combination therapy used as the initial regimen.

MATERIALS AND METHODS

Bacterial isolates. All four isolates for this study were obtained from the woman with native valve endocarditis described above. The timeline for these isolates and corresponding antibiotic therapies is presented in see Table 1 (below) and Fig. 1. The initial blood isolate in this study, W3148, was obtained on presentation of bacteremia at our institution, while the final blood isolate (W3248) was obtained after 2 weeks of consecutive daily positive blood cultures. The infection seeded to the subject's wrist, and two resulting isolates were obtained from this tissue 3 and 10 days (W3278 and W3348, respectively) after the final positive blood culture.

Antibiotics and media. Daptomycin was provided by the manufacturer (Cubist Pharmaceuticals, Lexington MA). Ceftaroline powder for *in vitro* analysis was kindly provided by Gary Doern with support of the manufacturer (Forest Laboratories, Inc., New York, NY), and oxacillin was purchased from Sigma-Aldrich, St. Louis, MO. Mueller-Hinton II broth (BD, Difco, Sparks, MD) supplemented with 50 µg/ml calcium (as CaCl₂) and 12.5 µg/ml magnesium (as MgCl₂) was used for all *in vitro* PK/PD analyses (MHB50). For any assays using oxacillin, this medium was further supplemented with 2% sodium chloride.

Susceptibility. MICs of daptomycin and vancomycin were determined in duplicate by Etest and broth microdilution, while ceftaroline and oxacillin susceptibilities were determined by broth microdilution (6). All samples were incubated at 35°C for 24 h. Daptomycin MICs were then determined in the presence of ceftaroline or oxacillin by placing a daptomycin Etest disc on Mueller-Hinton agar containing ceftaroline (0.5× MIC, at 0.5 µg/ml) or oxacillin (0.5 µg/ml or 0.5× MIC at 128 µg/ml) for each isolate (7).

PK/PD model. A previously described *in vitro* PK/PD model was used for simulating one-compartment antibiotic exposures of daptomycin and ceftaroline (1). This dynamic model allows for analysis of the pharmacodynamic effects of antibiotics under various simulated clearance conditions in humans (1, 25). All models were performed in duplicate experiments. The initial isolate, W3148, was grown overnight in 25 ml MHB50 to a 4.0 McFarland standard and added into the model (250 ml, total) to obtain a starting inoculum of 1×10^8 CFU/ml. Two monotherapy antibiotic regimens with free (f) drug concentrations were evaluated, using end-stage renal disease doses (9, 23) consistent with the doses used for the clinical subject and modeled over a 7-day (168-h) duration: (i) daptomy-

cin at 6 mg/kg every 48 h (targeted free maximum free drug concentration [fC_{max}], 8.0 µg/ml; half-life, 18.9 h) and (ii) ceftaroline at 200 mg every 12 h (targeted fC_{max} , 7.0 µg/ml; half-life, 6.2 h).

PK/PD modeling of combination therapy of antibiotics with two different elimination rates was performed according to the methods described by Blaser et al. (4). Briefly, the total elimination rate was set to that of the antibiotic with the faster clearance (ceftaroline). A supplemental dosing chamber was designed to continuously add antibiotic into the reaction (infection) chamber for the antibiotic that is normally cleared at the lower rate (daptomycin). Antibiotic doses were delivered into the supplement and reaction chambers for the slower-clearing antibiotic (daptomycin) to account for its elimination by the more rapid total rate. Combination therapy was simulated by two separate PD models: (iii) ceftaroline combined with daptomycin upon the first detection of daptomycin resistance with daptomycin therapy alone, and (iv) daptomycin plus ceftaroline initiated on day 1. In this fashion, we were able replicate antibiotic treatment from the clinical subject as well as determine the individual effects of these antibiotics.

Pharmacokinetic concentrations of daptomycin in test samples were evaluated by high-performance liquid chromatography (HPLC). The HPLC apparatus consisted of a Shimadzu controller SIL10A autosampler, LC10AD isocratic pump (Columbia, MO), and Gilson UV detector at 220 nm (Middleton, WI). The mobile phase was pumped through a Kinetix C₁₈ column (75 by 4.6 µm column; sub-2µm for breakthrough; Phenomenex, Torrance, CA) at a rate of 0.75 ml/min and consisted of 0.05 M formic acid adjusted to pH 3.0 and 35% acetonitrile. Aliquots (75 µl) of the standard and samples were injected directly without extraction or protein precipitation. Ceftaroline concentrations were evaluated by bioassay with *Bacillus subtilis* ATCC 6633 as the indicator organism and using standard drug concentrations of 1.0, 2.5, 5.0, and 10 µg/ml. The method for this assay has been described in detail elsewhere (28). The pharmacokinetic parameters were estimated and calculated using NONMEM modeling.

***In vitro* nonsusceptibility screening.** Susceptibility to daptomycin was evaluated at the start of each model and subsequently every 24 h during the simulation (29). Samples of 50 µl from the model were spot plated onto Mueller-Hinton agar plates containing 3 µg/ml daptomycin (3× the starting MIC). Plates were then examined for growth, and CFU/ml were quantified after 48 h of incubation at 35°C. Both Etest and broth microdilution methods were used to determine the MICs related to any resulting growth on screening plates.

Membrane integrity. Staphylococcal membrane permeability after antibiotic exposure was assayed with the LIVE/DEAD BacLight kit (Invitrogen, Madison, WI) (3). *In vivo*- and *in vitro*-derived isolates were grown in MHB50 and resuspended to an optical density at 600 nm (OD₆₀₀) of 0.25 in 5 mM HEPES buffer plus 50 µg/ml Ca²⁺. Daptomycin exposures were derived from the known C_{max} values for each dose, as follows: 6 mg/kg (8 µg/ml), 8 mg/kg (10 µg/ml), 10 mg/kg (12 µg/ml), and 12 mg/kg (16 µg/ml). Suspensions were incubated for 3 h at room temperature, brought to reagent concentrations of 5 µM SYTO-9 and 30 µM

TABLE 1 Antibiotic susceptibilities in clinical isolates from the study case

Isolate	Description	Day of DAP CPT combination therapy	MIC ($\mu\text{g/ml}$) ^a			DAP/VAN with CPT 0.5 $\mu\text{g/ml}$ ^b	OXA ^c	DAP/VAN with OXA 128 $\mu\text{g/ml}$ ^d	DAP/VAN with OXA 0.5 $\mu\text{g/ml}$ ^d
			DAP ^b	VAN ^b	CPT ^c				
W3148	Initial blood culture	-6	0.75-1.0	2.0	1.0	0.06/0.5	256	0.25/0.75	0.38/1.5
W3248	Final blood culture	4	2.0	3.0	1.0	0.06/0.5	256	0.06/1.0	0.25/1.5
W3278	Initial seeded wrist culture	7	0.13	1.5	1.0	0.06/0.5	256	0.06/1.5	0.13/1.5
W3348	Final seeded wrist culture	14	0.19	1.5	1.0	0.06/0.5	256	0.13/0.75	0.13/1

^a Abbreviations: DAP, daptomycin; VAN, vancomycin; CPT, ceftaroline; OXA, oxacillin.

^b Determined by Etest.

^c Determined by broth microdilution.

^d Values represent DAP and VAN Etest results after incorporation of CPT or OXA into the MHA medium.

propidium iodide, and incubated for an additional 15 min at room temperature in the dark. Fluorescence was measured in a Molecular Devices SpectraMax M2^e spectrofluorometer.

Membrane fluidity. Staphylococcal membrane fluidity was determined for *in vivo*- and *in vitro*-derived strains as previously described (2, 5). Overnight bacterial cultures were inoculated into fresh MHB50 medium and grown to an OD₆₀₀ of 0.2 to 0.5. Bacteria were pelleted, washed with normal saline, resuspended to an OD₆₀₀ of 0.3 in normal saline plus 10 μM 1,6-diphenyl-1,3,5-hexatriene (DPH), and incubated at 37°C for 1 h. Aliquots were transferred to preheated cuvettes, and fluorescence was measured in an ISS Koala spectrofluorometer with a temperature-controlled cuvette holder maintained at 37°C. The probe was excited with vertically polarized light (λ_{ex} , 360 nm), and emission intensity was detected in both parallel and perpendicular planes (λ_{em} , 426 nm). Results were corrected by subtracting data from an unlabeled control reaction. The polarization index (*pI*) is the sensitivity ratio of the instrument for detecting DPH emission and was determined using the following formula: $pI = [I_V - I_H(I_{HV}/I_{HH})]/[I_V + I_H(I_{HV}/I_{HH})]$, where *I* is the fluorescence intensity and *V* and *H* indicate vertical and horizontal orientation instrument values, respectively (2). Data represent averages \pm standard deviations of 3 experiments performed on separate days. A lower fluorescence polarization value indicates a higher degree of membrane integrity.

spa typing and multilocus sequence typing. The *spa* typing was performed following the method of Koreen et al. (15). The *spa* type identification was done using the Ridom StaphType (version 2.1.1) software (Ridom GmbH, Wurzburg, Germany). The multilocus sequence typing (MLST) was done as described by Enright et al. (10).

Statistical analysis. Comparisons were conducted by using Student's *t* test. For multiple comparisons within each measurement, reported *P* values are Holm adjusted.

RESULTS

***In vitro* antibiotic susceptibility.** The antibiotic susceptibility results of the study isolates are shown in Table 1. These strains belong to *spa* type t002 and MLST sequence type 5. The initial isolate, W3148, was susceptible to daptomycin, vancomycin, and ceftaroline, while the final blood culture was nonsusceptible to both daptomycin and vancomycin by Etest but remained susceptible to ceftaroline. The daptomycin MIC for this strain was 1 mg/liter (susceptible), and the vancomycin MIC was 4 mg/liter by broth microdilution. The secondary cultures obtained from the wrist were susceptible to each of these antibiotics by both Etest and broth microdilution. The daptomycin and vancomycin MICs in the blood culture isolates were lower (4- to 32-fold reductions) in the presence of both ceftaroline and oxacillin at 0.5 \times the respective MICs. A greater reduction in daptomycin MICs occurred in the presence of these antibiotics, compared to the change in vancomycin MICs. At an equivalent concentration of 0.5 $\mu\text{g/ml}$, ce-

taroline reduced daptomycin MICs up to 32-fold, compared to up to an 8-fold reduction with oxacillin.

Simulated antibiotic exposures in the PK/PD model. An *in vitro* pharmacodynamic model of renal failure (creatinine clearance, <30 ml/min) was utilized to simulate the *in vivo* antibiotic regimen of the patient. Daptomycin at 6 mg/kg every 48 h was modeled until the emergence of daptomycin nonsusceptibility, defined as an absolute rise in the MIC to a level of 2 $\mu\text{g/ml}$, and then ceftaroline at 200 mg every 12 h was added in combination for the remainder of the 7-day duration. Alternative regimens were also modeled to compare these effects to single antibiotic therapy or combination antibiotic therapy for the entire duration. The pharmacokinetic results of daptomycin and ceftaroline from the PK/PD model are displayed in Fig. 2 and were consistent with the range of values for the representative doses in patients with renal failure: for daptomycin, fC_{max} , $7.6 \pm 1.1 \mu\text{g/ml}$; fC_{min} , $0.8 \pm 0.3 \mu\text{g/ml}$; $t_{1/2}$, 18.5 h; area under the concentration-time curve for the free drug from 0 to 24 h ($fAUC_{0-24}$), 110.8 $\mu\text{g/ml} \cdot \text{h}$; for ceftaroline

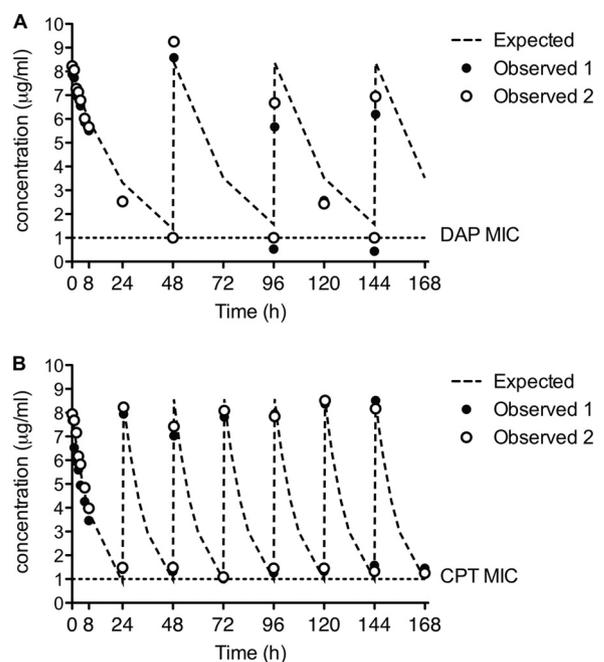


FIG 2 Pharmacokinetic concentration-time profiles of expected and observed concentrations in the one-compartment *in vitro* pharmacodynamic model over the 7-day simulation. (A) Daptomycin; (B) ceftaroline.

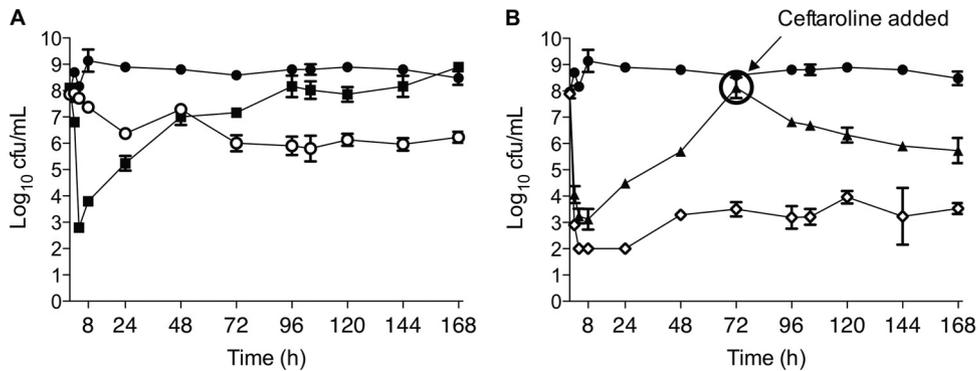


FIG 3 Antibiotic activity in the *in vitro* PK/PD model of daptomycin alone and in combination with ceftaroline in the initial daptomycin-susceptible clinical isolate W3148. (A) Single antibiotic exposure. black circles, growth control; black squares, daptomycin at 6 mg/kg every 48 h; white circles, ceftaroline at 200 mg every 12 h. (B) Combination antibiotic exposure results. Black circles, growth control; black triangles, DAP treatment every 48 h with CPT added on day 3; white diamonds, initial therapy with DAP every 48 h combined with CPT on day 1.

line, fC_{max} , $8.2 \pm 0.2 \mu\text{g/ml}$; fC_{min} , $1.1 \pm 0.3 \mu\text{g/ml}$; $t_{1/2}$, 4.3 h; 98% of the time above the MIC, and 36% of the time above $4 \times$ the MIC.

Daptomycin alone at 6 mg/kg every 48 h was bactericidal in the model within the first 8 h of simulated therapy (mean maximum kill, $5.3 \pm 0.3 \log \text{CFU/ml}$). As demonstrated in Fig. 3A, bacterial regrowth was detected after 24 h of therapy and increased for the entire 7-day duration with this dose. Daptomycin resistance after the 6-mg/kg dose, indicated by a MICs of 2 to 4 $\mu\text{g/ml}$ by Etest and 2 $\mu\text{g/ml}$ by broth microdilution, was detected at 72 h of therapy and was the predominant organism phenotype at the end of the model simulation (Fig. 4). Following these results, we replicated daptomycin monotherapy for 3 days and then added ceftaroline into the model to simulate combination therapy in the patient. The results in Fig. 3B demonstrate that the effects of daptomycin alone with this strain for up to 72 h were reproducible, and combining ceftaroline with daptomycin after the emergence of daptomycin resistance resulted in enhanced bacterial killing. This did not result in bactericidal activity ($2.4 \pm 0.8 \log \text{kill}$), but the burden of organisms at the end of therapy with this regimen at 7 days was significantly lower than with daptomycin therapy alone (mean, 5.7 ± 0.5 versus $8.9 \pm 0.2 \log \text{CFU/ml}$, respectively; $P <$

0.001). Daptomycin resistance occurred in each model of daptomycin monotherapy but varied in the initiation and degree of formation up to 72 h (Fig. 4). Notably, within 72 h after the introduction of ceftaroline combination therapy in the model, daptomycin resistance could no longer be detected. Isolates recovered during the last 24 h of daptomycin plus ceftaroline therapy had MICs of $\leq 0.75 \mu\text{g/ml}$ by Etest and $\leq 0.5 \mu\text{g/ml}$ by broth microdilution, which are equivalent to or less than the starting MIC of 1 $\mu\text{g/ml}$ for the wild-type strain.

Cefaroline in combination with daptomycin was also simulated as initial therapy in the PK/PD model (Fig. 3B). At least a 6.0-log decrease in inoculum was observed within the first 4 h of therapy, and this effect was maintained throughout the 7-day duration, with less than a 2-log regrowth by the end of therapy. These effects were greater than those observed with any other regimen tested. This initial combination therapy approach also inhibited the emergence of daptomycin resistance.

Membrane integrity with daptomycin. The effects of daptomycin concentrations on membrane integrity of the *in vivo*-derived strains are shown in Table 2. As expected, the wild-type daptomycin-susceptible parent strain exhibited a high degree of membrane damage when exposed to daptomycin conditions equivalent to peak concentrations with 6-mg/kg doses. No additional membrane damage was apparent with higher daptomycin exposures. Conversely, membrane integrity was relatively preserved with the 6-mg/kg and 8-mg/kg exposures in the resistant *in vivo* mutant. Higher-dose exposures of 10 mg/kg and 12 mg/kg produced membrane damage similar to that of the susceptible

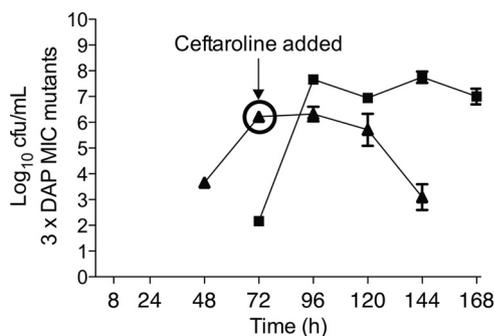


FIG 4 Emergence of daptomycin (DAP) resistance in the pharmacodynamic model detected after screening plates containing daptomycin at 3 $\mu\text{g/ml}$. Daptomycin resistance occurred in each model of daptomycin monotherapy, but it varied in the initiation and degree of formation up to 72 h. The addition of ceftaroline (CPT) at 72 h to the DAP regimen eliminated DAP resistance in model isolates. Black squares, DAP at 6 mg/kg every 48 h; black triangles, DAP at 6 mg/kg every 48 h with CPT added on day 3.

TABLE 2 Daptomycin cell membrane effects in isolates from the patient

Isolate	% of intact cell membranes after daptomycin exposure at ^a :				Membrane fluidity (pI) ^b
	6 mg/kg	8 mg/kg	10 mg/kg	12 mg/kg	
W3148	22.4 ± 4.2	25.7 ± 1.4	17.9 ± 1.7	18.3 ± 3.2	0.294 ± 0.018
W3248	43.0 ± 10.0	29.7 ± 1.4	16.9 ± 1.9	17.1 ± 3.1	0.288 ± 0.016
W3278	22.1 ± 4.2	20.6 ± 0.5	20.2 ± 1.3	18.5 ± 0.7	0.284 ± 0.020
W3348	32.7 ± 5.7	20.5 ± 1.4	19.5 ± 1.0	18.3 ± 0.5	0.287 ± 0.020

^a Values are means \pm standard deviations. A lower percentage of intact cell membranes indicates greater daptomycin activity.

^b A lower pI value indicates a greater degree of membrane fluidity.

parent strain following lower daptomycin exposures. The isolates obtained from the wrist during daptomycin plus ceftaroline clinical treatment displayed similar membrane integrity profiles to the initial parent strain.

Membrane fluidity. Daptomycin nonsusceptibility has been linked to changes in membrane fluidity (18). It is suggested that cationic peptides, including daptomycin and host peptides, exert maximal activity in the presence of an optimal or stable cell membrane order (30). A more fluid cell membrane may inhibit insertion of cationic peptides into the target cell membrane and has been associated with reduced cationic peptide susceptibility (17).

In comparing membrane fluidity between the daptomycin-susceptible parent strain and the *in vivo*-derived resistant mutant, the development of daptomycin resistance resulted in more-fluid cell membranes, although this difference was not statistically significant. *In vivo*, daptomycin susceptibility was restored in isolates W3278 and W3348 following ceftaroline combination therapy in the patient, but the enhanced fluidity persisted (Table 2).

Significant increases in membrane fluidity occurred in daptomycin-resistant isolates derived from the *in vitro* PK/PD model. The greatest increase in fluidity occurred soon after the emergence of daptomycin resistance (MIC, 3 $\mu\text{g/ml}$) at 96 h (pI , 0.281 ± 0.014 ; $P < 0.05$), but this change was not sustained in the final resistant isolate at 168 h (MIC, 4 $\mu\text{g/ml}$) from this regimen (pI , 0.286 ± 0.021 ; $P > 0.05$). Membrane fluidity at the end of ceftaroline combination exposure in the model appeared to return to level comparable to the initial isolate (pI , 0.289 ± 0.039).

DISCUSSION

This study presents a novel clinical case of microbiologically successful daptomycin and ceftaroline combination therapy following the emergence of daptomycin resistance. The noted clinical effects of combination in this case included sterilization of blood cultures following persistently positive daily cultures for 2 weeks with daptomycin monotherapy, reduction in the size of the cardiac vegetative infection source during combination therapy, and daptomycin resensitization by two susceptibility methods in genetically identical isolates that seeded to the patient's wrist during the endovascular infection. To our knowledge, this is the first reported case of improvement and restoration of daptomycin sensitivity *in vivo* with concomitant beta-lactam exposure.

In vitro pharmacodynamic modeling revealed microbiological findings similar to this case, including improved antibacterial activity after the addition of ceftaroline and the restoration of daptomycin susceptibility with combination therapy. Importantly, combination modeling of both antibiotics on the first day of simulated therapy displayed rapid and sustained bactericidal activity and prevented the emergence of daptomycin resistance. Although this study was limited to one clinical case and subsequent evaluation of a single clinical isolate in the model, we and others have successfully treated similar cases of complicated MRSA infections with this combination that were refractory and/or resistant to primary daptomycin therapy (G. Sakoulas, personal communication). The potent antimicrobial activity with an initial daptomycin-plus-ceftaroline combination treatment may be useful in complex MRSA infections, including isolates with daptomycin MICs approaching the susceptibility breakpoint of 1 $\mu\text{g/ml}$.

Ceftaroline is a new cephalosporin antibiotic with activity against a diverse range of Gram-negative and Gram-positive organisms, including MRSA and VISA (27). Ceftaroline activity

against MRSA is maintained through its affinity for the penicillin-binding protein 2a, which is responsible for resistance to other antistaphylococcal beta-lactams (27). Although ceftaroline alone is active against daptomycin-nonsusceptible strains in animal and *in vitro* models (13, 28), it is unknown if its added exposure improves daptomycin efficacy against these strains analogous to that found with other beta-lactam antibiotics (7, 11). Single-drug therapy with ceftaroline in the PK/PD model was bacteriostatic against the parent strain in our study over the 7-day duration, but antibiotic activity and susceptibility were further enhanced when ceftaroline was used in combination with daptomycin. We highlight that the ceftaroline dose used in this case and in simulation are based upon the regimen for its approved indications. More frequent dosing every 8 h has been successfully used as salvage therapy for bacteremia and endocarditis (12), and this may be desirable for complicated infections due to MRSA to optimize the pharmacodynamic parameter of time above the MIC (8).

A recent patient investigation of daptomycin in combination with antistaphylococcal beta-lactams for complicated MRSA infections demonstrated important microbiologic findings. Daptomycin susceptibility was restored upon *in vitro* exposure to nafcillin in a daptomycin-resistant strain that emerged while on therapy (7). Similar effects were noted in our study with both oxacillin and ceftaroline. Based upon susceptibility testing, a 32-fold reduction in the daptomycin MIC was observed with the *in vivo* daptomycin-resistant strain when tested in the presence of ceftaroline. Interestingly, ceftaroline overall had a 2- to 6-fold larger effect on lowering daptomycin MICs than did an equivalent concentration of oxacillin. We anticipate future studies comparing the effects of ceftaroline and oxacillin or nafcillin on daptomycin activity in pharmacodynamic models.

Enhanced daptomycin membrane binding and reduction in the net positive membrane surface charge are known to occur in the presence of beta-lactams (7, 26). Restoration of daptomycin susceptibility in our *in vitro* study correlated with a change in cell membrane fluidity near that of the parent isolate and may provide another mechanistic explanation for the phenotypic change in susceptibility. Prior investigations have shown a link between cell wall degradation and more rigid cell membranes (30). These findings support the hypothesis that cell wall damaging effects of ceftaroline in MRSA may lead to a compensatory more rigid cell membrane and thus restoration of daptomycin susceptibility. Although the changes in membrane fluidity were less apparent in our study, minor changes in cell membrane fluidity can have profound impacts on cell function and antibiotic susceptibility (14, 19).

Daptomycin exhibits concentration-dependent activity, and several *in vitro* and animal models have reported improved bactericidal activity with simulated doses ranging from 8 to 12 mg/kg, compared to 4 to 6 mg/kg daily (20, 24). High-dose therapies in these models particularly improve the efficacy of daptomycin against nonsusceptible strains compared to conventional doses of 6 mg/kg daily (20, 24). In a recent noncomparative and retrospective study of higher-dose daptomycin therapy for cases refractory to vancomycin treatment, doses of at least 8 mg/kg were both safe and effective for the treatment of complicated infections (16).

Although the daptomycin dose in this present case was increased to 10 mg/kg, this occurred only after the clearance of blood cultures with 6-mg/kg doses when combined with cef-

taroline. We did not assess this high-dose regimen against the initial blood isolate in the PK/PD model, but a membrane damage assay revealed that daptomycin concentrations did have notable effects on cell membrane integrity. As expected, the daptomycin-resistant isolate W3248 retained greater cell membrane integrity at exposures analogous to C_{max} for the 6-mg/kg and 8-mg/kg doses, indicating that daptomycin activity at these doses is reduced. Membrane damage was comparable between susceptible and resistant strains at higher-dose exposures of 10 mg/kg and 12 mg/kg. It would be interesting to discern if ceftaroline further enhances daptomycin activity at these higher doses in the *in vitro* model against strains with reduced daptomycin susceptibilities.

The patient's pharmacokinetic profile in this case was a challenge for direct pharmacodynamic modeling *in vitro*. One major limitation was to simulate daptomycin distributions during hemodialysis. During the two interdialytic periods with thrice-weekly hemodialysis, daptomycin is administered every 48 h for 2 doses and then every 72 h for one dose. For these reasons and due to the difficulty in mimicking 4-hour hemodialysis sessions, a severe renal dysfunction model (creatinine clearance of <30 ml/min) with every 48-hour dosing was used as a surrogate. Also, ceftaroline pharmacokinetic concentrations in the combination model with daptomycin could not be evaluated due to interactions with the microbiology organism, but this is a well-described method to appropriately simulate model combination therapies *in vitro* (4). Nonetheless, the microbiologic findings from the model correspond well with those noted during patient treatment. It is likely that similar activity relationships with daptomycin and ceftaroline occur under normal clearance conditions.

Although these results are promising, additional studies are needed to determine the ideal beta-lactam agent and doses to use in combination with daptomycin for refractory MRSA infections with reduced daptomycin susceptibility.

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