Clinical Therapeutics/Volume ▮, Number ▮, 2014

Antimicrobial Salvage Therapy for Persistent Staphylococcal Bacteremia Using Daptomycin Plus Ceftaroline

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ABSTRACT

Purpose: Guidelines recommend daptomycin combination therapy as an option for methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia after vancomycin failure. Recent data suggest that combining daptomycin with a β-lactam may have unique benefits; however, there are very limited clinical data regarding the use of ceftaroline with daptomycin.

Methods: All 26 cases from the 10 medical centers in which ceftaroline plus daptomycin was used for treatment of documented refractory staphylococcal bacteremia from March 2011 to November 2012 were included. In vitro (synergy studies, binding assays, cathelicidin LL-37 killing assays), and in vivo (virulence assays using a murine subcutaneous infection model) studies examining the effects of ceftaroline with daptomycin were also performed.

Findings: Daptomycin plus ceftaroline was used in 26 cases of staphylococcal bacteremia (20 MRSA, 2 vancomycin-intermediate *S aureus*, 2 methicillin-susceptible *S aureus* [MSSA], 2 methicillin-resistant S *epidermidis*). Bacteremia persisted for a median of 10 days (range, 3–23 days) on previous antimicrobial therapy. After daptomycin plus ceftaroline was started, the median time to bacteremia clearance was 2 days (range, 1–6 days). In vitro studies showed ceftaroline synergy against MRSA and enhanced MRSA killing by cathelicidin LL-37 and neutrophils. Ceftaroline also induced daptomycin binding in MSSA and MRSA to a comparable degree as nafcillin. MRSA grown in subinhibitory concentrations of ceftaroline showed attenuated virulence in a murine subcutaneous infection model.

Implications: Ceftaroline plus daptomycin may be an option to hasten clearance of refractory staphylococcal bacteremia. Ceftaroline offers dual benefit via synergy with both daptomycin and sensitization to innate host defense peptide cathelicidin LL37, which could attenuate virulence of the pathogen. (*Clin Ther.* 2014;▮:▮▮▮–▮▮▮) © 2014 Elsevier HS Journals, Inc. All rights reserved.

Key Words: ceftaroline, daptomycin, MRSA, MRSE bacteremia, MSSA, VISA.

Accepted for publication May 23, 2014.
http://dx.doi.org/10.1016/j.clinthera.2014.05.061
0149-2918/$ - see front matter

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INTRODUCTION

Bacteremia due to methicillin-resistant *Staphylococcus aureus* (MRSA) poses significant surgical and medical challenges to clinicians and the health care system.[1] The most difficult cases are those that persist despite appropriate antimicrobial therapy and without an easily identified and removable focus, or cases in which an infected biomedical device is identified but cannot be removed without extreme risks to the patient.[2] We have previously shown *S aureus* cross-resistance between cationic antimicrobial host defense peptides (HDPs) of the human innate immune system and vancomycin and daptomycin,[3,4] the only antibiotics approved by the US Food and Drug Administration for the treatment of MRSA bacteremia. These data portend a worrisome scenario in clinical cases in which the pathogen resists eradication and resistance to these agents could develop simultaneously under continuous selective pressures, not just by administered antibiotics but also by HDPs. Thus, the sense of clinical urgency in eradicating these infections is just becoming realized.

We have previously described very successful outcomes in patients with refractory MRSA bacteremia using combination therapy with daptomycin and an antistaphylococcal β-lactam.[5] In addition, development of daptomycin resistance by MRSA in vitro was suppressed in the presence of antistaphylococcal β-lactams.[6]

Ceftaroline was approved by the US Food and Drug Administration in 2010, and it became the first β-lactam available in the United States in 2011 with in vitro and in vivo MRSA activity for the treatment of bacterial skin and skin structure infections.[7] We anticipated that the combination of daptomycin plus ceftaroline therapy might exhibit superior activity against MRSA given the following: (1) the demonstrated synergy between daptomycin and β-lactams[5]; (2) the intrinsic activity of ceftaroline against MRSA[7]; (3) the observed decrease in *S aureus* ceftaroline MIC in *S aureus* upon loss of daptomycin susceptibility[8]; and (4) a recent case in which daptomycin plus ceftaroline was used successfully in salvage therapy with supporting in vitro data.[9]

In the present article, we report the use of daptomycin and ceftaroline as a salvage antimicrobial regimen in the treatment of refractory staphylococcal bacteremia at 10 US medical centers. In vitro synergy of ceftaroline plus daptomycin against MRSA is demonstrated and correlated to enhanced daptomycin binding induced by ceftaroline. Finally, we show that antistaphylococcal activity of human cathelicidin HDPs and neutrophils of the innate immune system are significantly increased by ceftaroline.

MATERIALS AND METHODS

Clinical Cases

All 26 cases from the 10 medical centers in which ceftaroline plus daptomycin was used for the treatment of documented refractory staphylococcal bacteremia from March 2011 to November 2012 were included (Figures 1 and 2). Additional case details are outlined in Table I. Participating institutions included the following: Sharp Memorial Hospital, San Diego, California; Detroit Medical Center, Detroit, Michigan; Oregon Health & Science University Hospital, Portland, Oregon; Westchester Medical Center, Valhalla, New York; University of Wisconsin Hospital, Madison, Wisconsin; The Ohio State University Wexner Medical Center, Columbus, Ohio; Dekalb Medical Center, Decatur, Georgia; Maimonides Medical Center, Brooklyn, New York; VA San Diego Healthcare System, San Diego, California; and John D. Dingell VA Medical Center, Detroit, Michigan. Approval or waiver from each center's institutional review board was obtained where appropriate based on the number of cases per site.

Bacterial Isolates

One MRSA isolate (SA1, case 23, isolate 2, daptomycin MIC 1.0–2.0 mg/L; ceftaroline MIC 1 mg/L; nafcillin MIC 8 mg/L) and 1 methicillin-susceptible *S aureus* (MSSA) isolate (LUC77, case 22, daptomycin MIC 1.0 mg/L; ceftaroline MIC 0.25 mg/L; nafcillin MIC 0.5 mg/L) available from the case series were chosen for further in vitro analyses. SA1 was determined to have a daptomycin MIC of 1 mg/L by using broth microdilution testing but an MIC of 2 mg/L by Epsilometer test (Etest, BioMerieux, Durham, NC). In vivo mouse studies described in the following text were performed on previously published strain MRSA Sanger 252 in which this infection model was well established in our laboratory.[10]

In Vitro Assays

Kill curves were performed in Mueller-Hinton broth supplemented with 50 mg/L Ca2+ by using a starting inoculum of 10^7 CFU/mL. Samples were obtained at 0, 4, 24, and (in some cases) 48 hours,

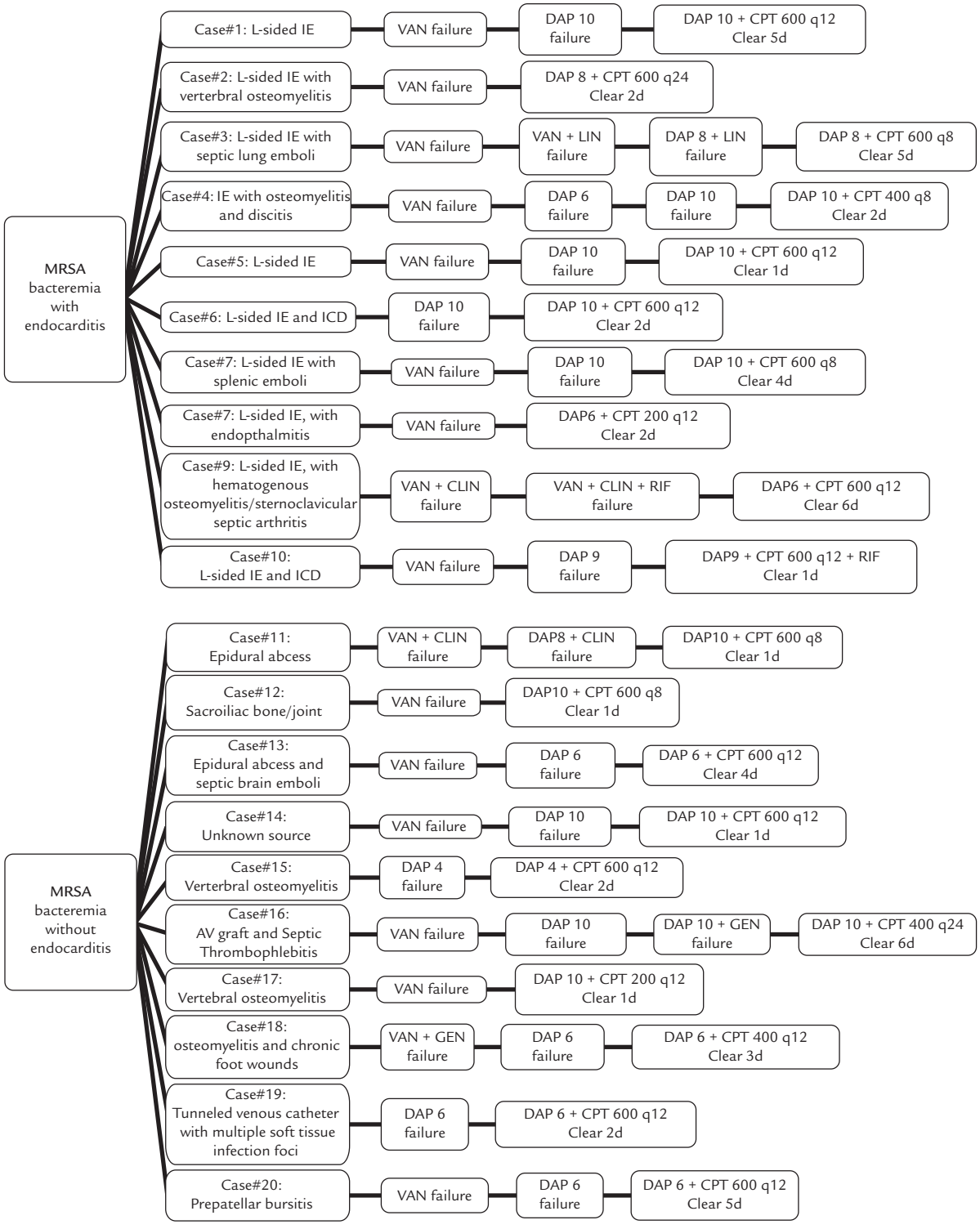

Figure 1. Summary of cases in which daptomycin (DAP) + ceftaroline (CPT) was used to clear persistent methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia, stratified according to the presence (top strata) or the absence (bottom strata) of endocarditis. Additional details can be found in the Table 1. L = left; IE = infective endocarditis; VAN = vancomycin; LIN = linezolid; ICD = infected cardiac device; RIF = rifampin; CLIN = clindamycin; GEN = gentamicin; AV = arteriovenous.

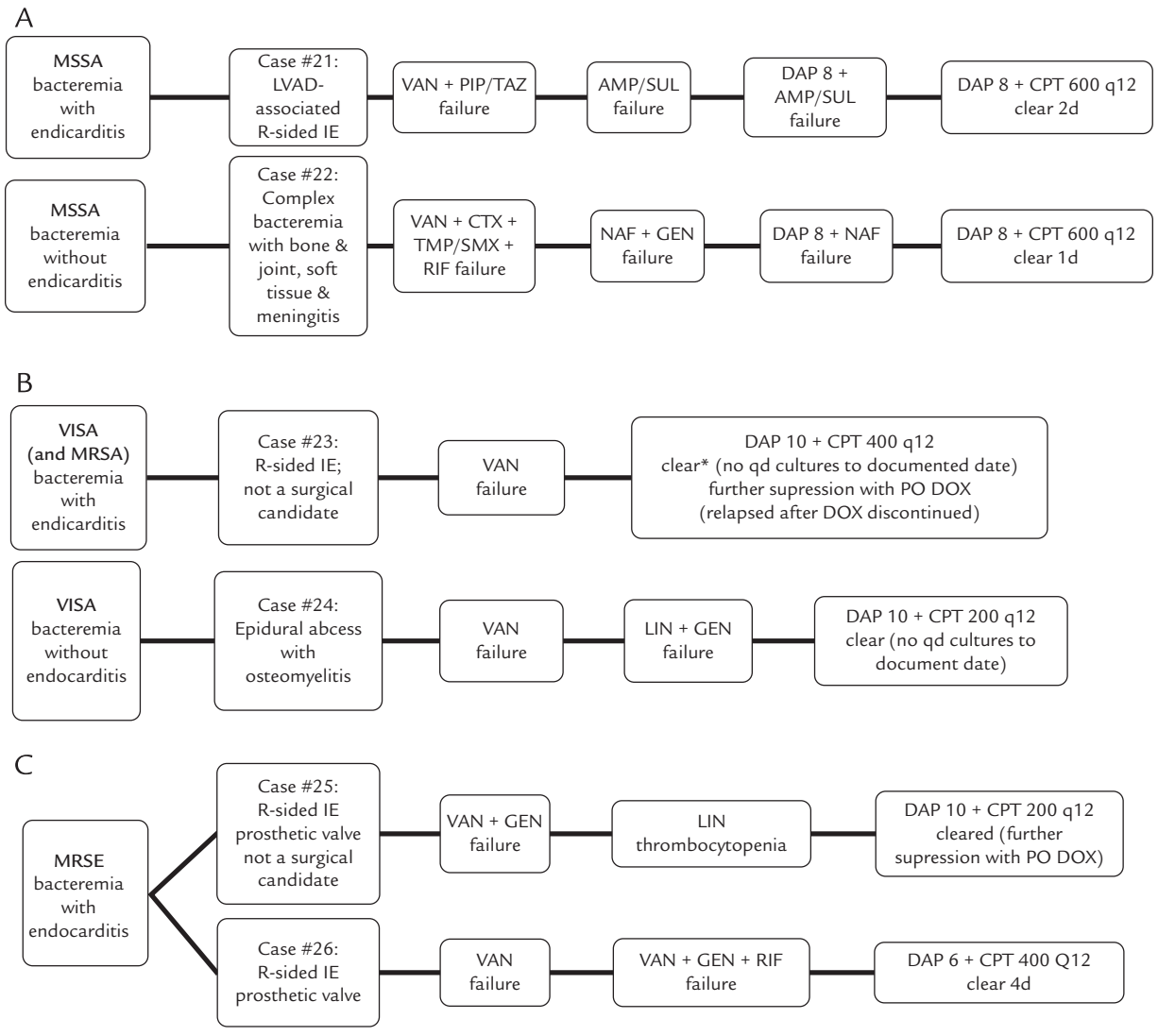

Figure 2. Summary of cases in which daptomycin (DAP) + ceftaroline (CPT) was used to clear either (A) methicillin-susceptible *Staphylococcus aureus* (MSSA), (B) vancomycin-intermediate-susceptible *S aureus* (VISA), or (C) methicillin-resistant S *epidermidis* (MRSE). Additional details can be found in the Table 1. Case 23: VISA infective endocarditis (IE) case relapsed with bacteremia and endophthalmitis after doxycycline (DOX) was discontinued; relapse was re-treated with DAP + CPT. LVAD = left ventricular assist device; L = left; VAN = vancomycin; PIP/TAZ = piperacillin/tazobactam; AMP/SUL = ampicillin/sulbactam; CTX = ceftriaxone; TMP/SMX = trimethoprim/sulfamethoxazole; RIF = rifampin; NAF = nafcillin; GEN = gentamicin; R = right; PO = oral; LIN = linezolid.

serially diluted 1:10 to $1:10^7$, and 10 mL plated in duplicate on Todd Hewitt agar (THA) plates. Assays were performed in duplicate in each experiment, and experiments were performed twice on separate days. Colonies were enumerated after 24 hours and $\log_{10}$ CFU/mL calculated for graphical presentation. One representative experiment is shown. Limit of detection was 1000 CFU/mL ($\log_{10} = 3$).

Susceptibility testing to daptomycin in varying concentrations of ceftaroline or nafcillin was performed by using broth microdilution methods established by the Clinical and Laboratory Standards Institute.[11]

Table I. Clinical details of cases included in this study.

Patient	Age/Sex Pathogen (MICs)	Comorbidities	Diagnostic Findings	Site(s) of Infection	Antimicrobial Therapies 1st Line	2nd Line	3rd Line	4th Line	Comments
MRSA cases									
1	44 M MRSA (VAN 2, DAP 1, CPT 0.5)	HIV/AIDS (CD4 count 0 Viral load >1 million), Afib	Mitral valve IE	L-sided IE	VAN d 1-3	DAP 10 d 4–9	DAP 10 + CPT 600 Q12 d 10–43 **Clear 5 d**	None	
2	75 M MRSA (VAN 2, DAP <0.5, CPT 2)	ESRD on HD, HTN, DM	AV 3 × 5 mm vegetation	IE (side unspecified) with discitis, osteomyelitis	VAN d 1	DAP 8 + CPT 600 q24 d 2–3 **Clear 2 d**	DAP 10 + CPT 600 q24 d 4	DAP 10 + LIN d 5-9	Changed to DAP monotherapy for 6 weeks once stabilized
3	67 M MRSA (VAN 1, DAP 0.25)	Asthma	MRSA bacteremia; EBV proliferative disorder; pulmonary septic emboli; TTE AV vegetation Splenic infarcts; sepsis	Aortic valve IE Pulmonary septic emboli	VAN d 1–5	VAN + LIN d 6–12	DAP 8 + LIN d 13–18	DAP 8 + CPT 600 q8 d 19-35 **Clear 5 day**	Changed to DAP 8 + VAN + MERO + CIPRO, and then DAP 8 + CPT 600 q12+ MOXI DAP stopped d 43 due to suspected pneumonitis (CT chest [ground glass infiltrates]) + peripheral eosinophilia Discharged on VAN + MOXI
4	69 M MRSA (VAN 2, DAP 0.25)	HTN, CKD, Obesity, DM, anemia of CKD, HTN, stroke, T6-L4 spinal fusion 1 month previous	Complicated by early MRSA surgical site infection with osteomyelitis/ hardware with MRSA bacteremia	Osteomyelitis and discitis IE	VAN d 1–2	DAP 6 d 3–6	DAP 10 d 7–9	DAP 10 + CPT 400 q8 d 10-17 **Clear 2 day**	Patient provided comfort care and died
5	51 M MRSA	CHF, DM, HTN, CKD, Afib	Echodensity along RV wire	Left-sided IE	VAN d 1–3	DAP 10 d 3–10	DAP 10 + CPT 600 q12 d 10–52 **Clear 1 d**	None	Pacer removed d 4; sent home on d 18 of DAP + CPT
6	73 M MRSA	Prostate cancer, HTN, DM, Afib, CHF	2.4-cm mass RV and RA leads	L-sided IE and ICD (pacemaker)	DAP 10 d 1–7	DAP 10 + CPT 600 q12 d 7–28 **Clear 2 d**	DAP 10 d 29–51	None	Pacemaker removed d 13
7	55 M MRSA (VAN 1.5, DAP 3)	Hepatitis C, IVDU, COPD, stroke	MV 0.7 X 1.3 cm vegetation	L-sided IE (MV) with splenic emboli	VAN d 1–7	DAP 10 d 8–11	DAP 10 + CPT 600 q8 d 12–72 **Clear 4 day**	None	Patient had MV replacement with prosthetic valve on d 29. Tissue valve culture was negative
8	43 F MRSA	ESRD on HD DM	Endophthalmitis	Presumed L-sided IE	VAN d 1–3	DAP 6 q48, CPT 200 q12 d 4–14 **Clear 2 d**	DAP 6 q48 d 15–28	None	Suspected initial source: HD catheter
9	47 M MRSA	DM, HTN, ETOH abuse	Complicated soft tissue infection Sternoclavicular septic arthritis	L-sided IE (MV) with hematogenous bone/joint	VAN + CLIN d 1–3	VAN + CLIN + RIF d 4–10	DAP 6 + CPT 600 q12 d 10–37 **Clear 6 d**	None	
10	86 M MRSA (VAN 2, DAP 1, CPT 1)	Afib, Dementia, HTN, GERD, venous stasis, glaucoma, blind	TEE negative, culture of pacemaker wires (+) MRSA VAN MIC 2	Presumed L-sided IE; infected pacemaker	VAN d 1–3	DAP 9 d 4–19	DAP 9+ CPT 600 q12+ RIF d 20–41 **Clear 1 day**	LIN d 41-48 DOX d 48-57	Pacemaker removed d 13; cleared bacteremia d 20 (7 d after pacemaker removal and 1 d after DAP + CPT); discharged on DOX PO

(continued)

Table I. (continued).

Patient	Age/Sex Pathogen (MICs)	Comorbidities	Diagnostic Findings	Site(s) of Infection	Antimicrobial Therapies				Comments
					1st Line	2nd Line	3rd Line	4th Line	
11	55 M MRSA (VAN 1, DAP <0.5)	DM, HTN	MRI epidural abscess 50 cm × 20 cm × 3 cm	Epidural abscess	VAN + CLIN d 1–3	DAP 8 + CLIN d 4–8	DAP 10 + CPT 600 q8 d 9–12 **Clear 1 d**	DAP 10 + TMP-SMX d 13-50	Abscess drainage d 2
12	27 M MRSA (VAN 2, DAP 1, CPT 1)	HIV with disseminated MAC, asthma	MRI of sacroiliac joint abscess 2.4 × 2 cm periarticular fluid; TTE negative	Septic sacroiliac joint with adjacent abscess	VAN d 1–9	DAP 10 + CPT 600 q8 d 10–29 **Clear 1 d**	None	None	
13	29 F MRSA	IVDU; anemia	Spinal MRI: T12-L3 epidural and paraspinal abscesses; Septic emboli to brain with resulting cranial nerve III, IV, and VI palsies; left pleural empyema	Bilateral psoas abscess and epidural abscess with septic brain emboli	VAN d 1	DAP 6 d 2–3	DAP 6 + CPT 600 q12 d 4–19 **Clear 4 d**	VAN d 20-62	VAN × 6 wk was started at discharge
14	76 M MRSA (VAN 2, DAP ≤0.5)	CAD, DM, HTN, ESRD on HD COPD, morbid obesity	TEE negative MRI/CT negative	Unknown source	VAN d 1	DAP 10 d 2–7	DAP 10 + CPT 600 q12 d 7–17 **Clear 1 d**	DAP 10 d 18-56	
15	63 M MRSA (VAN 2, DAP 2)	DM, morbid obesity Ventral hernia with colon necrosis/ perforation; previous PICC-associated MRSA bacteremia	CT: Psoas and *iliopsoas* abscesses, vertebral osteomyelitis TEE negative	Vertebral osteomyelitis	DAP 4 d 1–2	DAP 4+ CPT 600 q12 d 3–5 **Clear 2 d**	LIN d 6–10	CPT 600 q12 d 11-42	DAP 4 based on actual body weight, DAP 6 based on ideal body weight
16	66 F MRSA (VAN 1, DAP ≤0.5, CPT 0.5)	HIV, DM, HTN, ESRD on HD, CHF, asthma, HCV	TEE negative, US of LUE: heterogeneous echogenic foci noted	IV catheter; AV graft and septic thrombophlebitis	VAN d 1–2	DAP 10 d 3–5	DAP 10 + GEN 1 mg/kg d 6–7	DAP 10 + CPT 400 q24 d 8-22 **Clear 6 d**	Source of bacteremia unknown for 10 days; Removal of AV graft d 11; cleared 72 h after graft removal. De-escalated to DAP 10 as outpatient treatment for 6 wk with HD
17	60 M MRSA (VAN 2, DAP 1)	IVDU admitted for NSTEMI, AKI, and septic shock, HD	TEE negative; CT scan small thigh abscess; MRI of spine lumbar osteomyelitis without epidural abscess	Small abscesses in the right thigh; Osteomyelitis of C2–C6	VAN d 1–7	DAP 10 + CPT 200 q12 d 8–14 **Clear 1 d**	DAP 10 d 15–53	None	Patient had AKI and septic shock; started HD on day 8 of admission; De-escalated to DAP 10
18	63 M MRSA	ESRD on HD, DM	Chronic wounds on feet with osteomyelitis	HD catheter	VAN + GEN × 5 d 1–5	DAP 6 × 5 d d 6–10	DAP 6 + CPT 400 q 12 d 11–26 **Clear 3 d**	None	Recent 6 wk previous course of VAN
19	63 F MRSA	T-cell lymphoma	Multiple skin infection foci	Venous access tunneled venous catheter	DAP 6 d 1–5	DAP 6 + CPT 600 q12 d 6–15 **Clear 2 d**	VAN d 16–22	LIN d 23-28	
20	49 M MRSA	DM, HTN, BPH	No other foci	Prepatellar bursitis	VAN d 1–8	DAP 6 d 9–13	DAP 6 + CPT 600 q12 d 14–21 **Clear 5 d**	CPT 600 q12 d 22–42	

(continued)

Table I. (continued).

Patient	Age/Sex Pathogen (MICs)	Comorbidities	Diagnostic Findings	Site(s) of Infection	Antimicrobial Therapies 1st Line	2nd Line	3rd Line	4th Line	Comments
MSSA cases									
21	50 M MSSA	Nonischemic cardiomyopathy LVAD 3 y previously	TEE negative CT abdomen: No abscess	LVAD infection Wound culture: MSSA/ *Escherichia coli* Blood culture: MSSA only	VAN + PIP/TAZ d 1–3	AMP/SUL d 3–5	DAP 8 + AMP/SUL d 5–6	DAP 8+ CPT 600 q12 d 6-12 **Clear 2 day**	Patient signed out AMA on d 12 of therapy; given a prescription for PO cephalexin + RIF; Returned with *E coli* bacteremia twice (1 mo and 3 mo later); died 4 mo later (MSSA bacteremia had never returned)
22	54 M MSSA	DM, hepatitis C, ETOH abuse	TEE-negative CT/MRI: Retroperitoneal Phlegmon, L3–L5 Discitis/osteomyelitis/epidural abscess. Psoas pyomyositis CSF: 2186/mm^3 WBC (89% PMN), Glucose 31 mg/dL, protein 240 mg/dL	Retroperitoneal infection	VAN/CTX/TMP-SMX/RIF d 1-3	NAF + GEN d 3–6	DAP 8 + NAF d 6–10	DAP 8+ CPT 600 q12 d 10-17 **Clear 1 d**	Treated purely with medical therapy DAP 8 + NAF d 17–24 NAF d 24–56 Complete cure 6-mo follow-up
VISA cases									
23	60 F VISA/MRSA (isolate 1: VAN 4, DAP 3, CPT 0.75) (isolate 2: VAN 2, DAP 1-2 [see text], CPT 0.75)	DM, HTN, s/pCABG Prior MRSA sternal wound Infection × 2	TEE: atrial appendage 33-mm thrombus NM scan: no increased focal uptake CT/MRI spine chest/abdomen/pelvis: no osteomyelitis, no abscess	RA appendage septic thrombus Poor surgical candidate	VAN d 1–30	DAP 10 + CPT 400 q12 42 days	PO DOX (noncompliant)	Retreatment for relapse	Discharge on suppression on DOX re-admitted with bacteremia and endophthalmitis after stopped taking DOX retreated with DAP + CPT and discharged on DOX
24	71 F VISA (VAN 3-4; DAP 2, CPT 0.38)	ESRD on HD s/p laminectomy	TEE negative US AV graft: no fluid collection CT spine: lumbar osteomyelitis, Discitis Epidural abscess	Epidural abscess Osteomyelitis	VAN	LIN + GEN	DAP 10 + CPT 200 q12 42 days	DOX 90 days	DOX × 3 months; no sign of recurrence LIN caused thrombocytopenia RIF resistance emerged
MRSE cases									
25	83 F MRSE (VAN 4, DAP 0.5, CPT 0.25)	ESRD on HD, CABG, AVR Cirrhosis Prior MRSE bacteremia 3 times in past	TEE negative, mild MV thickening US AV graft: no fluid collection NM scan: no increased focal uptake CT/MRI spine Chest/Abdomen/Pelvis: no osteomyelitis, no abscess	Probable IE	VAN + GEN d 1–9	LIN d 10–16	DAP 10+ CPT 200 q12 d 17–42 d	DOX Suppression therapy	LIN caused thrombocytopenia RIF resistant TMP-SMX severe allergy 2 prior breakthrough bacteremia on DOX and on wk 6 DAP Poor surgical candidate

(continued)

Table I. (continued).

Patient	Age/Sex Pathogen (MICs)	Comorbidities	Diagnostic Findings	Site(s) of Infection	Antimicrobial Therapies				Comments
					1st Line	2nd Line	3rd Line	4th Line	
26	60 M MRSE	ESRD on HD DM	AV, MV bioprosthetic valve endocarditis	Left-sided IE, prosthetic valve	VAN d 1–3	VAN + GEN+ RIF d 4-14	DAP 6 + CPT 400 q12 d 15–46 Clear 4 d	None	Suspected source: HD catheter

MRSA = methicillin-resistant *Staphylococcus aureus;* M = male; VAN = vancomycin; DAP = daptomycin; CPT = ceftaroline; IE = *infective endocarditis;* Afib = atrial fibrillation; ESRD = end-stage renal disease; HD = hemodialysis; HTN = hypertension; DM = diabetes mellitus; AV = arteriovenous; LIN = linezolid; *EBV* = Epstein-Barr virus; TEE = transes ophageal echocardiography; MERO = meropenem; CIPRO = *ciprofloxacin;* MOXI = moxifloxacin; CT = computed tomography; CKD = chronic kidney disease; CHF = congestive heart failure; RV = right ventricular; RA = right atrial; ICD = infected cardiac device; IVDU = intravenous drug use; COPD = chronic obstructive pulmonary disease; MV = mitral valve; F = female; ETOH = alcohol; RIF = rifampin; GERD = gastroesophageal reflux disease; *DOX* = *doxycycline;* MRI = magnetic resonance imaging; CLIN = clindamycin; TMP-SMX = trimethoprim-sulfamethoxazole; CAD = coronary artery disease; PICC = peripherally inserted central catheter; HCV = hepatitis C virus; US = ultrasound; GEN = gentamicin; NSTEMI = non-ST-segment elevation myocardial infarction; AKI = acute kidney injury; MSSA = methicillin-susceptible *S aureus;* LVAD = left ventricular assist device; *AMP/SUL* = *ampicillin/sulbactam;* PIP/TAZ = piperacillin/tazobactam; AMA = against medical advice; CSF = cerebrospinal fluid; WBC = white blood cell count; PMN = polymorphonuclear cell; CTX = ceftriaxone; VISA = vancomycin-intermediate *S aureus;* CABG = coronary artery bypass graft; NM = nuclear medicine; MRSE = methicillin-resistant *S epidermidis;* AVR = *aortic valve replacement.*

Human cathelicidin LL-37 susceptibility testing and killing assays were performed in RPMI media supplemented with 5% Luria broth (LB) as previously described.[5,12]

Daptomycin binding assays were performed, as previously described, after bacteria were grown to OD_{600nm} of 0.6, exposed for 1 hour with ceftaroline 1 mg/L or nafcillin 10 mg/L, and then labeled with 4 mg/L bodipy–daptomycin (Cubist Pharmaceuticals, Lexington, Massachusetts) for 15 minutes.[5,12] The antimicrobial activity of bodipy–daptomycin is slightly reduced such that the MIC is 1 dilution higher than unlabeled daptomycin. Cytochrome c binding was performed as previously described.[13] Ceftaroline was provided by Forest Pharmaceuticals (New York, New York).

Neutrophil Killing Assays

Neutrophils were freshly isolated from the blood of healthy donors by using the PolyMorphPrep kit (Fresenius Kabi, Homburg, Germany), and erythrocytes were lysed with sterile H_2O as previously described.[14] MRSA Sanger 252 was grown to log phase in ceftaroline 0.1 μg/mL or media alone (untreated), washed, and resuspended in phosphate-buffered saline (PBS). At this concentration of ceftaroline, there was no appreciable effect on growth rate. Bacteria were inoculated at a multiplicity of infection = 1 with 5×10^5 polymorphonuclear cells in RPMI + 2% 70°C heat-inactivated fetal bovine serum in suspension culture plates.[15] After 90 minutes of incubation at 37°C/5% CO_2, cells were lysed with 0.025% Triton X-100, and the total number of remaining bacteria were enumerated on THA plates. Survival was calculated as the percentage of the initial inoculum. Experiments were performed by using blood from at least 3 healthy donors. Use and procedures were approved by the University of California San Diego Human Research Protections Program.

Animal Model of MRSA Cutaneous Infection

MRSA Sanger 252 has been used in our laboratory to study the effect of β-lactam antibiotics on MRSA virulence. The strain was grown overnight to the stationary phase in 40 mL of antibiotic-free LB or LB containing ceftaroline 0.1 mg/L. Bacteria were washed in PBS 40 mL, and resuspended in 2 mL of PBS and 2 mL of Cytodex beads (Sigma-Aldrich, St. Louis, MO) (1 mg/L), yielding 10^{10} CFU/mL. Next, 0.1 mL of the bacterial suspension was injected subcutaneously into flanks of 25-g female CD1 mice.

To control for animal-to-animal variability, paired lesions were created, with ceftaroline-treated MRSA on one side and antibiotic-free grown MRSA on the contralateral side using a total of 12 mice. Right and left sides were alternated for antibiotic-free grown and ceftaroline-grown MRSA.

Lesion sizes were measured at 2 days and compared by using a paired-sample Wilcoxon signed-rank test. A P value of <0.05 was considered significant. All animal studies were performed under protocols that were reviewed and approved by the University of California San Diego Institutional Animal Use and Care Committee. All animal research in this investigation was performed in accordance with national and local guidelines that are in place to maximize humane animal treatment.

RESULTS

Case Series

Characteristics of the Clinical Cases

Daptomycin plus ceftaroline was used in 26 cases of staphylococcal bacteremia from 10 geographically and demographically distinct US hospitals. Clinical details are provided in Table I, and a summary of relevant clinical characteristics are provided in Table II. The pathogens consisted of MRSA in 20 cases (Figure 1), MSSA in 2 cases, vancomycin-intermediate *S aureus* (VISA) in 2 cases, and methicillin-resistant *S epidermidis* in 2 cases (Figure 2). As expected, endocarditis (confirmed echocardiographically) was highly represented (54% [14 of 26]), and included 12 patients with left-sided, 1 side unspecified, and 1 right-sided endocarditis. The mean patient age was 60 years, and 73% were males. Nine (35%) of the patients had end-stage renal disease and were on hemodialysis, and 15 (58%) patients had diabetes mellitus.

Daptomycin Plus Ceftaroline Salvage

The daptomycin plus ceftaroline combination was used most often as third-line therapy in 13 cases (50%), followed by second-line therapy in 8 cases (31%), and fourth-line therapy in 5 cases (19%). The median duration of daptomycin plus ceftaroline combination therapy was 16 days. Once deemed stable, some patients received additional de-escalated, step-down antibiotics replacing daptomycin plus ceftaroline (eg, daptomycin monotherapy, oral doxycycline), to complete a median total duration of 42 days of therapy for staphylococcal bacteremia (Tables I and II). Twenty-five (96%) of the patients survived completion of therapy; 1 patient died when medical care was withdrawn due to multiple medical problems upon which the MRSA bacteremia was superimposed.

The use of daptomycin plus ceftaroline combination therapy was mostly for documented failure of bacteremia clearance. For 23 of the 26 cases for which detailed microbiologic information was available via serial daily blood culture results, bacteremia persisted for a median of 10 days (range, 3–23 days) on previous antimicrobial therapy, and the bacteremia cleared in a median of 2 days (range, 1–6 days) after daptomycin plus ceftaroline was started. It is critical to note that in all but 1 case (case 21; Table I), the bacteremia clearance on daptomycin plus ceftaroline therapy was not temporally related to a surgical procedure such as device removal.

Endocarditis Cases

As anticipated from a case series examining salvage therapy for staphylococcal bacteremia, a large percentage of patients (14 of 26 [54%]) had bacterial endocarditis. These cases warrant closer scrutiny because bacteremia persistence is a salient feature of their clinical and microbiologic course. These 14 patients comprised 10 with MRSA, 1 with MSSA, 1 with VISA, and 2 with methicillin-resistant *S epidermidis* endocarditis. Daptomycin plus ceftaroline was used as second-line therapy for 4 patients, third-line therapy for 7, and fourth-line therapy for 2. Among the 10 patients with endocarditis who had serial blood cultures collected, bacteremia cleared a median of 2 days (range, 1–6 days) after daptomycin plus ceftaroline therapy was started.

Nonsusceptible Organisms

Other salient features in these cases are the involvement of nonsusceptible organisms to daptomycin in 4 cases and ceftaroline in 1 case. The ceftaroline-resistant isolate was successfully cleared after 2 days with the combination therapy using daptomycin 8 mg/kg per day plus ceftaroline 600 mg every 24 hours. The cases with daptomycin nonsusceptibility involved: (1) 2 cases with VISA (cases 23 and 24); (2) one case with 7 days of vancomycin therapy followed by daptomycin 10 mg/kg (case 7); and (3) 1 case treated with daptomycin 6 mg/kg based on ideal body weight that was 4 mg/kg actual body weight (case 15).

Table II. Summary of patient and infection characteristics. Values are given as median (range) or number (%).

Characteristic	Value (n = 26)
Age, y	60 (27–86)
Male	19 (73)
Pathogen	
MRSA	20 (77)
MSSA	2 (8)
VISA	2 (8)
MRSE	2 (8)
Common comorbidities	
Diabetes	15 (58)
Hemodialysis	9 (35)
HIV/AIDS	3 (12)
Liver disease	5 (19)
Malignancy	2 (8)
Sites of infection*	
Endocarditis	14 (54)
Left-sided IE	12 (46)
Right-sided IE	1 (4)
Side not specified	1 (4)
Left ventricular assist device	1 (4)
Pacemaker/defibrillator	2 (8)
Osteoarticular	11 (42)
Discitis/vertebral osteomyelitis/epidural abscess	8 (31)
Sternoclavicular septic arthritis	1 (4)
Sacroiliac joint	1 (4)
Osteomyelitis and chronic foot wounds	1 (4)
Other deep tissue	3 (12)
Tunneled venous catheter with soft tissue infection foci	1 (4)
AV graft with septic thrombophlebitis	1 (4)
Prepatellar bursitis	1 (4)
Septic brain emboli/meningitis	2 (8)
Unknown bacteremia source	1 (4)
Bacteremia duration before DAP + CPT, d	10 (3–23)
Bacteremia duration after DAP + CPT, d	2 (1–6)
DAP + CPT salvage	
Second-line	8 (31)
Third-line	13 (50)
Fourth-line	5 (19)
Daptomycin dosing, mg/kg	
4	1 (4)
6	7 (27)
>8	18 (69)
CPT dosing	
q8 h	5 (19)
q12 h	19 (73)
q24 h	2 (8)
Duration of DAP + CPT, d	16 (3–61)
>7	23 (88)
>28	7 (28)
Duration of DAP + CPT plus follow-up antibiotics, d	42 (8–132)
>14	25 (96)
>42	14 (54)
Antimicrobial resistance	
Daptomycin nonsusceptible	4 (15)

MRSA = methicillin-resistant *Staphylococcus aureus;* MSSA = methicillin-susceptible *S aureus;* VISA = vancomycin-intermediate *S aureus*; MRSE = methicillin-resistant *S epidermidis*; IE = *infective endocarditis*; AV = *arteriovenous;* DAP = daptomycin; CPT = ceftaroline.
*May have >1 site, so total > 100%.

Refractory MSSA Bacteremia

Of note, 2 of the 5 cases in which the combination therapy was used as fourth-line therapy involved persistent bacteremia due to MSSA that failed to clear promptly with daptomycin in combination with nafcillin or ampicillin/sulbactam. One case (case 22; Table I) involved a massive retroperitoneal phlegmon localized between the vertebral spine posteriorly and the descending aorta anteriorly. The patient's bacteremia resolved, and he made a complete clinical, microbiologic, and radiographic recovery without any surgical intervention. The MSSA from this patient (LUC77) was subjected to in vitro study (described later). The second of the MSSA bacteremia cases warrants mention because the patient had an infected nonremovable left ventricular assist device, with superficial cultures growing MSSA and *Escherichia coli* and persistent blood culture specimens growing MSSA (case 21). The patient ultimately cleared the MSSA bacteremia after 8 days, including 48 hours on ceftaroline plus daptomycin. However, the patient had a history of medical noncompliance and abusive behavior toward medical staff, eliminating his chances for device exchange, and he signed himself out of the hospital against medical advice 4 days after bacteremia clearance (12 days of total parenteral antimicrobial therapy). He was prescribed cephalexin plus rifampin orally with unknown compliance. Remarkably, while the patient was readmitted 2 subsequent times with *E coli* bacteremia and concomitant

left ventricular assist device driveline infection, the MSSA bacteremia never recurred for 4 months until the patient died.

Antibiotic-related Adverse Effects

Characteristic adverse effects related to specific antimicrobial agents requiring alternative therapy were observed among patients in this case series. These adverse effects included hepatotoxicity and interstitial nephritis from rifampin, thrombocytopenia from linezolid, eosinophilic pneumonitis from daptomycin, and hypersensitivity reactions from trimethoprim/sulfamethoxazole.

In Vitro Synergy Testing

To determine in vitro synergy between daptomycin and ceftaroline, checkerboard (Table III) and time-kill studies were performed against MSSA LUC77 from case 22 (Figure 3A) and MRSA SA1 from case 23 (Figure 3B). Against MSSA LUC77, nafcillin 20 mg/L and ceftaroline 5 mg/L alone achieved comparable bactericidal killing at 24 hours. These concentrations were chosen because they approximate the free C_{max} achieved in vivo with standard dosing regimens.[7,16,17] When co-incubated with a subinhibitory daptomycin concentration of 0.5 mg/L, the ceftaroline combination produced further killing than ceftaroline alone, but the nafcillin combination allowed significant regrowth. Against MRSA, the combination of daptomycin 1 mg/L plus ceftaroline 0.1 mg/L demonstrated considerable and obvious synergy over each drug alone.

Table III. Daptomycin (DAP) susceptibilities of methicillin-resistant *Staphylococcus aureus* (MRSA) SA1 and methicillin-susceptible *S aureus* (MSSA) LUC77 in broth media containing varying concentrations of ceftaroline or nafcillin.

Antibiotic in Broth Media	LUC 77 (MSSA) DAP MIC (mg/L)	SA1 (MRSA) DAP MIC (mg/L)
Ceftaroline, mg/L		
0	1	2
0.125	0.25	1
0.25		0.5
0.50		0.125
1.0		
Nafcillin, mg/L		
0	1	2
0.125	0.5	2
0.25	0.25	2
0.5		2
1.0		2
2.0		2
4.0		1
8.0		

Daptomycin Binding

Our previous studies have demonstrated β-lactam–induced binding of daptomycin to MRSA[5] and vancomycin-resistant *Enterococcus faecium*.[12] Figure 4 microscopically and quantitatively demonstrates that ceftaroline also induces daptomycin binding in MSSA and MRSA comparably to nafcillin. Under these conditions of ceftaroline exposure, there was no significant difference in cytochrome c binding for either MRSA SA1 or MSSA LUC77, suggesting that the mechanism of enhanced daptomycin binding was not mediated by reduction in net surface charge (data not shown).

Ceftaroline Effects on Innate Staphylocidal Immunity

We have demonstrated that antistaphylococcal β-lactams and ampicillin enhance killing of MRSA[5] and vancomycin-resistant *E faecium*,[12] respectively, by cationic antimicrobial peptides. MRSA SA1 and MSSA LUC77 demonstrated MIC to LL-37 of 16 μM. In the presence of 0.1 mg/L of ceftaroline or nafcillin, the LL-37 MIC was reduced to 8 μM for both isolates. Figure 5A shows that against MRSA SA1, growth in subinhibitory concentrations of ceftaroline results in a concentration-dependent hypersensitization to killing by the human cathelicidin LL-37 (16 μM). We then examined the effects of ceftaroline on killing of MRSA Sanger 252 by using freshly isolated human neutrophils, which produce LL-37 abundantly. These assays demonstrated a significant enhancement of neutrophil killing of MRSA after the growth in ceftaroline 0.1 mg/L compared with the same strain grown in antibiotic-free LB broth (Figure 5B).

MRSA Sanger 252 was cultured under the same conditions and subsequently injected subcutaneously into CD1 mice. The exact inoculum was 2.95×10^9

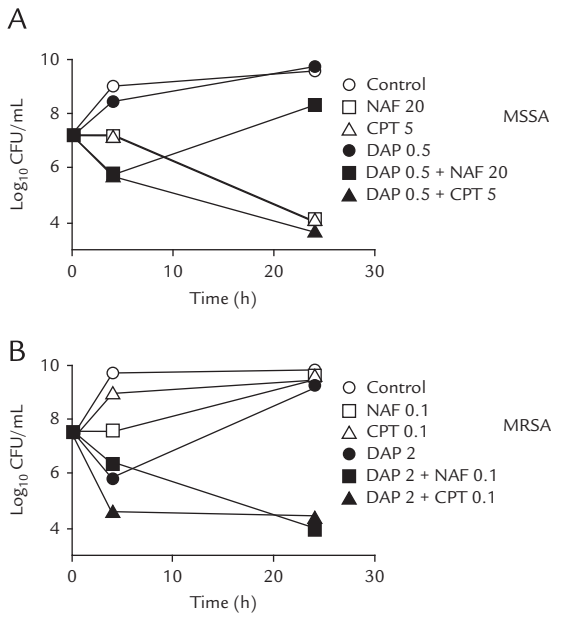

Figure 3. Kill curves demonstrating the effect of daptomycin (DAP), nafcillin (NAF), and ceftaroline (CPT) alone or in combination at the specified concentrations (milligrams per liter) against (A) methicillin-susceptible *Staphylococcus aureus* (MSSA) LUC77 and (B) methicillin-resistant *S aureus* (MRSA) SA1.

CFU for the control antibiotic-free grown MRSA and 2.90×10^9 CFU for the ceftaroline-grown MRSA in 0.1 mL, confirming minimal growth differences of MRSA Sanger 252 under these conditions. If the enhanced LL-37 and white blood cell activity rendered by exposure to ceftaroline was functionally significant in vivo, then attenuation in lesion sizes would be expected. Indeed, this outcome was observed as shown in Figure 5C, with MRSA Sanger 252 grown in ceftaroline 0.1 mg/L before mouse challenge producing significantly smaller lesion sizes compared with the same strain cultured in antibiotic-free media and injected on the contralateral side.

DISCUSSION

Refractory MRSA bacteremia is a very challenging clinical situation, especially when source control is not possible by virtue of an elusive or irremovable focus. Although such refractory infections have been appreciated to pose increased risks of adverse individual patient outcomes such as mortality and increased health care resource utilization, recent emerging data suggest more global implications of such infections.[1,2] It has long been appreciated that selection pressure on bacteria in a persistent high-inoculum focus of infection (particularly by suboptimally dosed antibiotics) can drive antibiotic resistance and therefore highlights the importance of surgical source control of such infections.[18,19] However, it is becoming increasingly apparent that antimicrobial resistance in staphylococci does not emerge in a closed system driven only by the antimicrobial agents themselves. We have shown that important cationic HDPs of the human innate immune system can select for resistance to vancomycin[3] and daptomycin[4] that clinicians subsequently administer to patients for therapy. The longer these infections persist, HDPs plus administered antibiotics may co-select drug-resistant bacteria if the bacterial inoculum remains high. A more recent study has even shown the emergence of daptomycin resistance in vivo under HDP selection pressure without the additional selective pressure of antibiotic therapy.[20] Although clearing these infections in the fastest possible manner reduces the risk of antibiotic resistance, the data are limited identifying the best possible approaches to achieve this goal.

Based on previous data,[21–25] we have examined and shown that the addition of antistaphylococcal β-lactams to daptomycin may prove to be helpful in bacteremia clearance.[5] In addition, β-lactams seem to reduce the development of daptomycin (and therefore possibly HDP) resistance in *S aureus*.[6] Through research collaborative discussions of this phenomenon, we identified centers where the novel cephalosporin ceftaroline was used in combination with daptomycin. These centers contributed all their cases in which the daptomycin plus ceftaroline combination was used as treatment of persistent staphylococcal bacteremia to this series.

As in the previous case series, the combination of daptomycin plus ceftaroline was highly successful, clearing the bacteremia in a median of 2 days after persisting for a median of 10 days. Although there is no way of knowing how this time to bacteremia clearance would compare with either agent alone (particularly with ceftaroline monotherapy), 2 very recent studies suggest that the combination therapy may clear bacteremia more rapidly than monotherapy. One study showed a mean

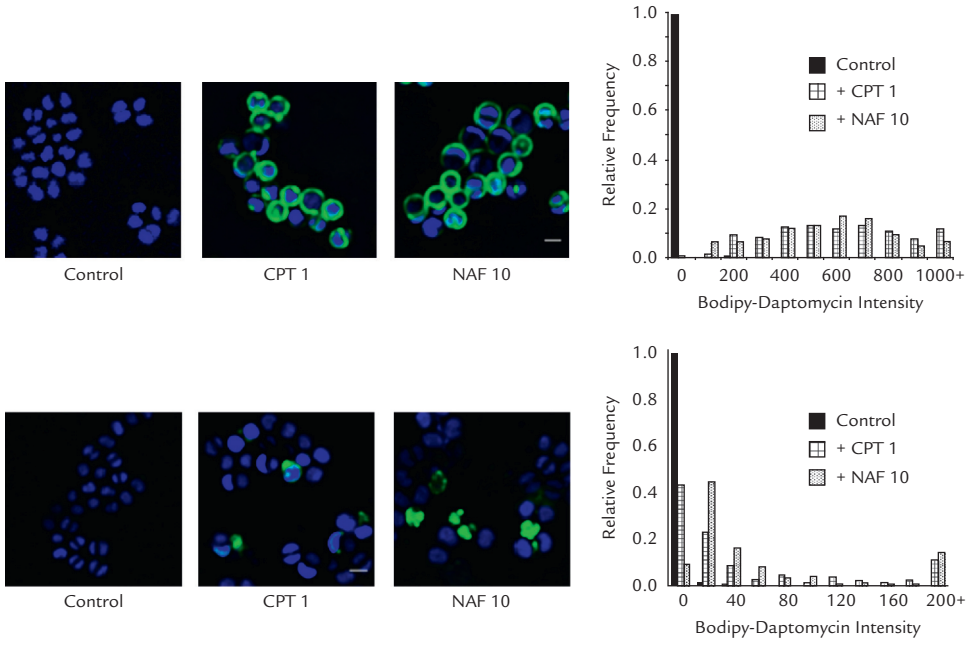

Figure 4. Effect of bodipy-labeled daptomycin (4 mg/mL) binding to methicillin-resistant *Staphylococcus aureus* isolate SA1 (top panel) or methicillin-susceptible *S aureus* isolate LUC77 (bottom panel) after exposure for 1 hour to either ceftaroline (CPT) 1 mg/L or nafcillin (NAF) 10 mg/L compared with no antibiotic. The accompanying histograms on the right quantitatively demonstrate that CPT and NAF result in higher intensity binding.

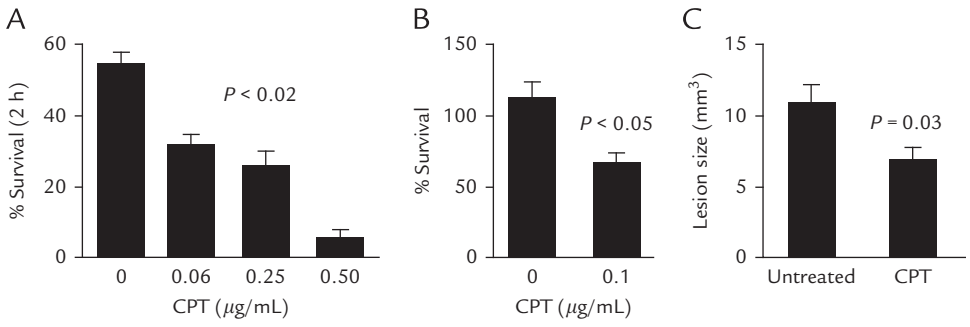

Figure 5. (A) Methicillin-resistant *Staphylococcus aureus* (MRSA) SA1 grown in antibiotic-free Luria broth (LB) or LB containing 0.06, 0.25, and 0.5 mg/L of ceftaroline (CPT), washed in phosphate-buffered saline, and subjected to LL37 16 μM. Percent survival at 2 hours in shown (Kruskal-Wallis ANOVA, $P = 0.02$). (B) CPT-sensitized MRSA Sanger 252 is more susceptible to killing by neutrophils. MRSA Sanger 252 grown in 0.1 mg/L of CPT is more susceptible to polymorphonuclear cell killing than bacteria grown in media containing no antibiotic (Mann-Whitney *U* test, $P < 0.05$). (C) CPT-sensitized MRSA Sanger 252 produces smaller lesions when injected subcutaneously into CD1 mice compared with the same strain grown in antibiotic-free media (paired-sample Wilcoxon signed-rank test, $P = 0.03$).

bacteremia duration of 5 days when salvage ceftaroline monotherapy was used in staphylococcal bacteremia after glycopeptide failure.[26] In another recent study that examined outcomes of 31 patients with persistent MRSA bacteremia treated with ceftaroline, 0% failure (0 of 10) was reported among the 10 patients who received ceftaroline combination therapy (5 with daptomycin), compared with 38% failure (8 of 21) among patients who received ceftaroline monotherapy (Fisher exact text, $P = 0.0317$).[27]

Against a MRSA from 1 case in our study, in vitro synergy was clearly demonstrated between daptomycin and ceftaroline. When evaluating MSSA from another case, synergy was found between daptomycin and ceftaroline; however, the addition of subinhibitory concentrations of daptomycin to nafcillin proved less effective in killing than nafcillin alone. This finding may potentially explain why nafcillin plus daptomycin failed to clear the bacteremia as a third-line regimen in MSSA bacteremia, yet the combination of daptomycin and ceftaroline was subsequently successful as a fourth-line regimen. It also highlights the complex pharmacodynamic interactions of antibiotics in vivo, with potential negative effects of combination antibiotic therapy against susceptible organisms. Although daptomycin plus nafcillin has shown great promise in the treatment of MRSA bacteremia, this combination needs to be examined further against MSSA. Based on previous work, we hypothesized that the differences between nafcillin and ceftaroline with respect to synergy with daptomycin rests on relative binding to penicillin-binding protein 1 (PBP1).[28] Ceftaroline seems to bind PBP1, as well as all other PBPs of *S aureus* with the exception of PBP4.[29] However, there is considerable heterogeneity in the 50% inhibitory concentrations on PBP1 and other PBPs between different *S aureus* strains, and these relative differences need to be explored further, likely through sequencing on PBP genes of different strains.

Treatment of the strains with ceftaroline 1 mg/L resulted in an enhancement of daptomycin binding to MRSA SA1 and MSSA LUC-77 comparable to nafcillin 10 mg/L (**Figure 4**). Interestingly, we did not see this effect when we reduced ceftaroline to 0.1 mg/L and grew the bacteria overnight. Thus, we "pulsed" the bacteria with ceftaroline 1 mg/L or nafcillin 10 mg/L for 1 hour and searched for changes in daptomycin binding, with no appreciable effect on bacterial viability. This finding suggests that binding to PBPs by ceftaroline must be >0.1 mg/L to produce the necessary physiologic changes that will result in daptomycin synergy, consistent with previous data on binding affinity of ceftaroline for *S aureus* PBPs.[29]

With respect to the enhancement of innate immune-mediated staphylocidal activity, however, growing bacteria overnight in ceftaroline 0.1 mg/L was sufficient to show enhancement of cathelicidin LL-37 killing, enhancement of neutrophil killing, and reduced virulence in skin lesion generation when injected into mice. We suspect that the reduced lesion sizes were due to the increased effectiveness of the host cathelicidin and neutrophil defenses in bacteria exposed to ceftaroline.

This study provides encouraging data to clinicians treating serious infections due to staphylococci, particularly cases of MRSA bacteremia refractory to standard forms of therapy due to unidentifiable or unremovable foci of infection. In addition, although the combination of daptomycin plus antistaphylococcal β-lactams has shown successful outcomes in a few cases of refractory MRSA bacteremia and substantiated by recent additional in vitro studies, one clinical case in this series of MSSA bacteremia and subsequent in vitro studies in this report suggest that this combination may be less potent than the antistaphylococcal agent alone for some MSSA strains, as seen for strain LUC77 (case 22; **Table I**). However, ceftaroline plus daptomycin exhibited excellent activity against this particular organism in vitro, and this combination was ultimately successful in clearing the bacteremia. The patient ultimately was de-escalated to daptomycin plus nafcillin and then to cefazolin monotherapy to complete a prolonged parenteral course of therapy, with an excellent clinical outcome. Thus, it seems that once an infection is adequately controlled, and there are no outstanding surgical management issues, it may not be necessary to complete an entire parenteral course of antimicrobial therapy with daptomycin plus the β-lactam or ceftaroline, as combination therapy can be very cumbersome and expensive outside an acute care hospital setting. However, we caution against early de-escalation in patients with left-sided endocarditis for which a cardiac surgical indication remains despite bacteremia clearance, and we suggest continuation of combination therapy until the patient can be bridged to surgery (G.S., unpublished data).

The present study is clearly limited in its retrospective, noncomparative design. Furthermore, although

bacteremia cleared a median of 2 days after daptomycin plus ceftaroline therapy, it is impossible to know how long the bacteremia would have taken to clear had the previous therapies been continued without reliance on daptomycin plus ceftaroline salvage. This question could only have been answered in a prospective comparative study. In addition, follow-up of >3 months was available for only 2 of the 25 surviving patients, and the durability of this therapy is unknown.

Nevertheless, there are promising data to suggest a clinical utility of daptomycin plus ceftaroline in the acute period to quench refractory bacteremia and potentially widen the time window to obtain prompt source control and reduce HDP-driven resistance to daptomycin and vancomycin. The medical centers that contributed cases to this series included all cases in which daptomycin plus ceftaroline was used, not just cases with successful outcome. This understanding, along with our companion in vitro studies, suggests that the combination of daptomycin and ceftaroline warrants further investigation. In more global terms, this study, as well as numerous previously in vitro,[21–24,28] animal models,[25] and human data,[5,26,27] suggest that daptomycin plus β-lactam therapy may be an appropriate candidate for formal large-scale clinical trials. Although direct comparisons of the current MRSA bacteremia treatment standards daptomycin and vancomycin monotherapy versus these agents with nafcillin added are probably the most anticipated among infectious disease clinicians, exploration of ceftaroline in combination with these agents is also warranted, probably as a follow-up study. We believe that combination therapy using β-lactams is poised to represent the new treatment paradigm for MRSA bacteremia in the future.

CONCLUSIONS

Ceftaroline plus daptomycin may be an option to hasten clearance of refractory staphylococcal bacteremia. Ceftaroline offers a dual benefit via synergy with both daptomycin and sensitization to innate host defense peptide cathelicidin LL37, which could attenuate virulence of the pathogen.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institute of Health, Division of National Institute of Child Health and Human Development (grant U54 HD071600-01 [September 26, 2011–June 30, 2016] Developmental and Translational Pharmacology of Pediatric Antimicrobial Therapy (Drs. Sakoulas and Nizet)]. Additional funding was provided by Forest Pharmaceuticals (New York, NY).

G. Sakoulas and P.A. Moise was responsible in searching of the literature. G. Sakoulas, P.A. Moise and V. Nizet was responsible in figure creation. G. Sakoulas and V. Nizet was responsible in designing the study. G. Sakoulas, A.M. Casapao, P. Nonejuie, J. Olson, C.Y.M. Okumura, M.J. Rybak, R. Kullar, A. Dhand, W.E. Rose, D.A. Goff, A.M. Bressler, Y. Lee, J. Pogliano, S. Johns, G.W. Kaatz and J.R. Ebright was responsible in data collection. G. Sakoulas, P.A. Moise and V. Nizet was responsible in data interpretation. And all the authors were responsible for writing of this paper.

CONFLICTS OF INTEREST

Dr. Sakoulas has received research grant support from Cubist Pharmaceuticals and speaking honoraria from Cubist, Pfizer, Forest, Novartis, and Astellas Pharmaceuticals. Dr. Moise is an employee and shareholder of Cubist. Dr. Casapao has received grant support from Cubist, Forest, and the Michigan Department of Community Health. Dr. Rybak has received grant support from, consulted for, or provided lectures for Cubist, Forest, Durata, Cepheid, and Novartis. Dr. Kullar is an employee and shareholder of Cubist; this research was conducted during her prior employment at Oregon State University. At that time, Dr. Kullar received speaking honoraria from Cubist and Forest, and served on the advisory board of Optimer. Dr. Dhand has received speaker's honoraria from Cubist and Pfizer. Dr. Rose has received grant support and speaking honoraria from Cubist and is a consultant for The Medicines Company and Visante, Inc. Dr. Goff has received grant support from Merck and serves on the advisory board of Optimer, Cubist, Merck, Rempex, and Forest. Dr. Pogliano has received research grants and consulting fees from Cubist. Dr. Nizet was formerly on the advisory board of Trius Therapeutics (acquired by Cubist). The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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