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Treatment of High-Level Gentamicin-Resistant *Enterococcus faecalis* Endocarditis with Daptomycin plus Ceftaroline

George Sakoulas,[a] Poochit Nonejuie,[b] Victor Nizet,[a] Joseph Pogliano,[b] Nancy Crum-Cianflone,[c] Fadi Haddad[d]

Department of Pediatric Pharmacology and Drug Discovery[a] and Department of Biology,[b] University of California San Diego School of Medicine, La Jolla, California, USA; Division of Infectious Diseases, Naval Medical Center San Diego, San Diego, California, USA[c]; Sharp Grossmont Hospital, La Mesa, California, USA[d]

A recurrent case of left-sided endocarditis caused by high-level aminoglycoside-resistant *Enterococcus faecalis* was successfully treated with ceftaroline and daptomycin. This combination demonstrated excellent synergy *in vitro*. Mechanistically, ceftaroline enhanced binding of daptomycin to the cell membrane and sensitized *E. faecalis* to killing by human cathelicidin LL-37, a cationic innate host defense peptide. Daptomycin plus ceftaroline may be considered in salvage therapy in *E. faecalis* endovascular infections and requires further study.

A 63-year-old man with a past medical history significant for hypertension presented with fevers for 1 month. The patient received levofloxacin and doxycycline for presumed prostatitis. Physical examination revealed a grade 2 systolic murmur and grade 1 diastolic murmur. Blood cultures were positive for *Enterococcus faecalis*. The patient was admitted to the hospital and started on ampicillin-sulbactam and gentamicin. The white blood cell count (WBC) was 10,100 cells/mm^3, hemoglobin was 14 g/dl, and the chest X-ray was normal. Repeat blood cultures showed ampicillin-susceptible *E. faecalis* with high-level gentamicin resistance (HLGR). A transesophageal echocardiogram revealed a 5-mm vegetation on the noncoronary cusp of the aortic valve. On the third hospital day, gentamicin was discontinued and ceftriaxone at 1 g intravenously (i.v.) every 12 h (q12h) was started, along with ampicillin at 2 g i.v. q4h. Blood cultures became negative after 96 h of treatment. The patient remained asymptomatic thereafter, and blood cultures remained negative during and after 6 weeks of therapy.

Two weeks after completion of therapy, the patient presented to the emergency department with a temperature of 39.2°C. Examination revealed a grade 3 systolic heart murmur and grade 1 diastolic murmur. A transesophageal echocardiogram showed severe aortic regurgitation and an increase in the size of the vegetation to 10 mm. *E. faecalis* was recovered from blood cultures without any change from the previous susceptibility profile. Ampicillin at 12 g, continuous infusion over 24 h, and ceftriaxone at 1 g i.v. q12h were started initially. On hospital day 2, ceftriaxone was switched to daptomycin at 8 mg/kg i.v. daily, based on prior data showing synergy between these antibiotics against enterococci and successful clinical use (1, 2). The patient became afebrile after 24 h of therapy. Blood cultures that were repeated after 48 and 96 h of daptomycin plus ampicillin therapy turned positive for the same isolate after 4 and 3 days, respectively.

Based on unpublished *in vitro* observations in our laboratory, which have demonstrated synergy between daptomycin and ceftaroline against several clinical bloodstream isolates of *E. faecalis* and *Enterococcus faecium*, and a published report of synergy between daptomycin and ceftaroline against MRSA (3), ampicillin was discontinued and ceftaroline at 600 mg i.v. every 8 h was added to the daptomycin treatment, and there was successful clearance of the bacteremia. The patient was discharged on a regimen of daptomycin at 8 mg/kg i.v. daily and ceftaroline at 600 mg i.v. every 8 h, and he was readmitted after 2 weeks for elective aortic valve replacement. Preoperative blood cultures were negative. Aortic valve tissue culture grew *E. faecalis* with high aminoglycoside resistance only from broth. Daptomycin plus ceftaroline therapy was continued for 4 weeks after surgery, and blood cultures obtained 1 week after completion of therapy were negative. The patient was deemed cured 6 weeks after completion of therapy.

TABLE 1 Reduction of daptomycin MIC in Mueller-Hinton broth supplemented to 50 mg/liter Ca^{2+} and containing incrementally higher concentrations of ceftaroline or ampicillin[a]

AMP or CPT (mg/liter)	DAP MIC (mg/liter) in presence of:	
	CPT	AMP
0	2	2
0.5	0.5	2
1.0	0.5	2
2.0	0.5	2
4.0	0.5	2
8.0	0.5	0.5
16.0	0.5	
32.0	0.25	

[a] DAP, daptomycin; CPT, ceftaroline; AMP, ampicillin.

Based on this excellent clinical and microbiological response, we performed checkerboard assays and determined kill curves at clinically relevant antibiotic concentrations (4–7) in Mueller-Hinton broth supplemented to 50 mg/liter Ca^{2+} to assess the synergy of daptomycin and ceftaroline against the relapse *E. faecalis* isolate from this patient. Daptomycin, ampicillin, ceftaroline, and ceftriaxone MICs were 2, 16, >32, and >32 mg/liter, respectively. The organism was qualitatively negative for beta-lactamase production by nitrocefin disk test. The checkerboard assay showed a

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Address correspondence to George Sakoulas, gsakoulas@ucsd.edu.

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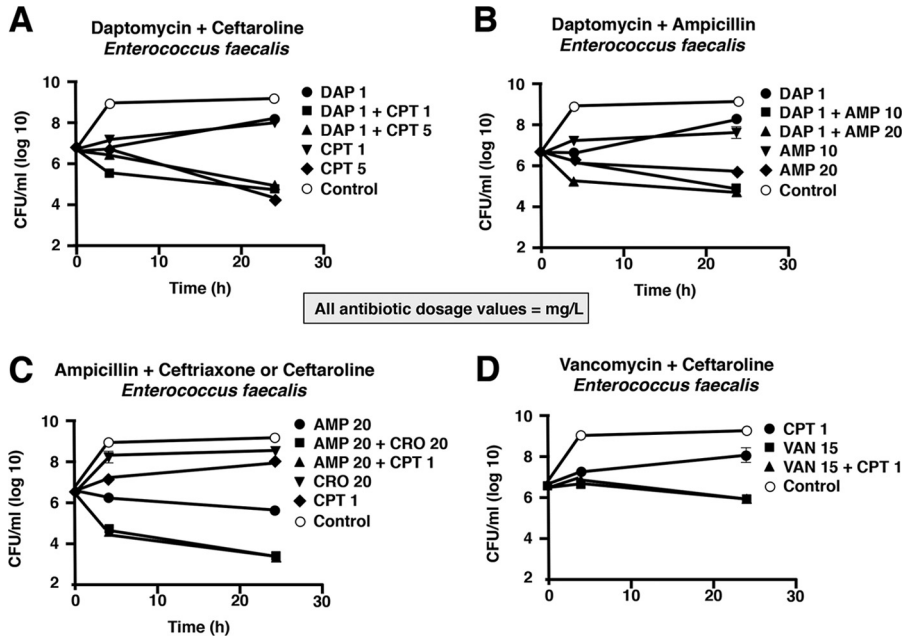

FIG 1 (A and B) Time-kill assays (24 h) in Mueller-Hinton broth supplemented to 50 mg/liter Ca^{2+}, evaluating the activity of daptomycin (DAP) alone or with ceftaroline (CPT) (A) or ampicillin (AMP) (B) against *E. faecalis*. (C and D) Results of similar experiments, showing an effect of AMP with either ceftriaxone (CRO) or CPT (C) and vancomycin (VAN) with CPT (D) against *E. faecalis*. Data are means of three experiments, with duplicate plating in each experiment. The limit of detection was 3.0 $\log_{10}$ CFU/ml.

4-fold reduction in the daptomycin MIC with ceftaroline at 0.5 to 16 mg/liter and ampicillin at 8 mg/liter (Table 1). No differences in the MIC were observed in checkerboard studies between ampicillin and ceftaroline or ampicillin and ceftriaxone.

Kill curve assays with daptomycin at 1 mg/liter plus ceftaroline at 1 or 5 mg/liter confirmed synergy, as had been observed in prior data with other clinical isolates, which prompted selection of this combination for this patient (Fig. 1A). In order to provide a context for this degree of killing with this combination compared to other regimens clinicians consider, we performed similar assays to determine relative synergy of daptomycin and ampicillin (Fig. 1B), ceftriaxone or ceftaroline with ampicillin (Fig. 1C), and vancomycin and

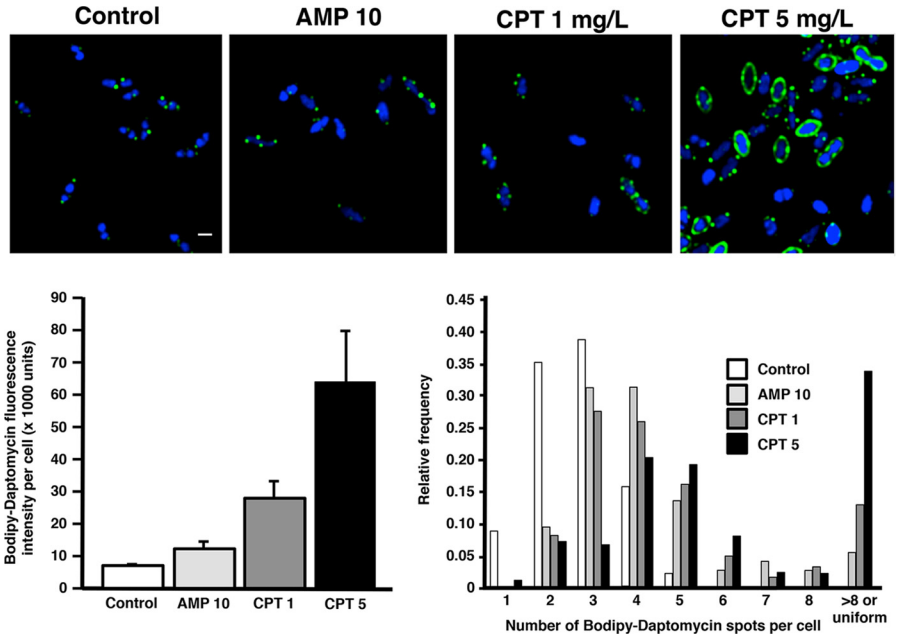

FIG 2 *E. faecalis* labeled with bodipy-daptomycin (16 mg/liter; 4× MIC; baseline MIC, 4 mg/liter) in LB broth for 15 min after a 45-min treatment with either ampicillin (AMP) at 10 mg/liter or ceftaroline (CPT) at 1 mg/liter or 5 mg/liter, compared to control untreated cells. The normalized total intensity of signal per cell (bottom left) and number of binding spots per cell (bottom right) are shown. Microscopy method details have been described elsewhere (2, 8).

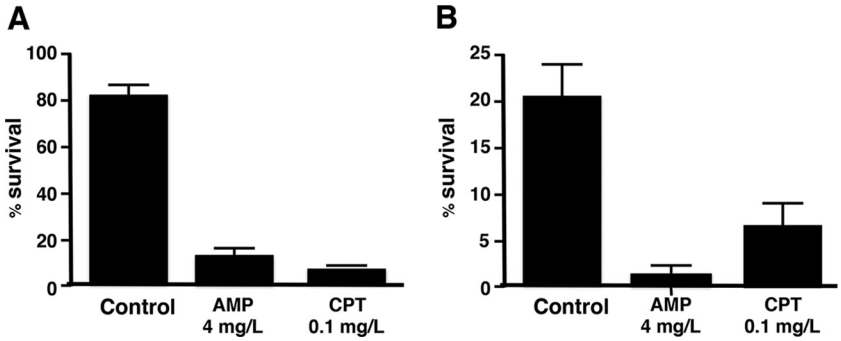

FIG 3 Percent survival of *E. faecalis* after 2 h of exposure to 64 μM (A) or 128 μM (B) cathelicidin LL37 in untreated controls compared to cells grown overnight in either ampicillin (AMP) at 4 mg/liter or ceftaroline (CPT) at 0.1 mg/liter. Method details have been described previously (2).

ceftaroline (Fig. 1D). These experiments showed the following: (i) bacteriostatic activity of vancomycin at 15 mg/liter and ampicillin at 20 mg/liter alone against this isolate, as anticipated; (ii) comparable synergy with ampicillin at 20 mg/liter and either ceftriaxone at 20 mg/liter or ceftaroline at 1 mg/liter; (iii) a lack of synergy for ceftaroline with vancomycin.

In agreement with our previous studies, which showed that ampicillin enhanced the binding of daptomycin to ampicillin-resistant *E. faecium* (2), using previously published methods (2, 8), growth of the present *E. faecalis* isolate in broth media containing either ampicillin at 10 mg/liter or ceftaroline at 1 or 5 mg/liter resulted in significantly increased daptomycin binding to the bacterial membrane compared to control bacteria grown in antibiotic-free LB broth (Fig. 2).

Also similar to what we had observed with *E. faecium* (2), growth of this *E. faecalis* strain in ampicillin or ceftaroline resulted in increased susceptibility to human cathelicidin LL-37 killing at 64 and 128 μM (Fig. 3). Note that this strain was much more susceptible to ampicillin and ceftaroline than the previously described *E. faecium*, and therefore much lower concentrations of drugs were used to allow growth under experimental conditions. Interestingly, this *E. faecalis* strain was much more resistant to cathelicidin LL-37 (MIC, 64 μM) than we observed for *E. faecium* (MIC, 8 μM), with both isolates from patients with endocarditis. This pattern may represent another interesting reflection of the β-lactam–antimicrobial peptide susceptibility seesaw effect across the enterococcal species, and it is a potential area for further study regarding the differences in endovascular pathogenicity between *E. faecium* and *E. faecalis*.

Assessment of surface charge with or without ceftaroline or ampicillin in cytochrome *c* binding assays showed no significant differences in this property (data not shown), which is perhaps an indication of the lack of significant surface charge effects when low concentrations of β-lactams are used.

This is the first case demonstrating a successful clinical outcome with use of daptomycin plus ceftaroline in a case of *E. faecalis* endocarditis, with supporting *in vitro* data demonstrating synergy between these drugs against *E. faecalis* and enhancement of cathelicidin peptide activity and daptomycin binding by ceftaroline. We point out that the ceftriaxone dose utilized initially was lower than that recommended in the literature and may have led to treatment failure (9). While limited to a single case, these results point to several alternative avenues of therapy that need to be studied clinically for the treatment of serious enterococcal endovascular infections. Treatment of these infections can be hampered by the lack of a validated bactericidal monotherapy, as shown in this case, and intrinsic and acquired antimicrobial resistance in *E. faecium* superimposed on many host comorbidities. In treating *E. faecalis* endocarditis, use of ampicillin and gentamicin appears straightforward in treatment guidelines (10). However, in the practical clinical world, when not limited by HLGR as in this case, the otovestibular toxicity, nephrotoxicity, and therapeutic drug monitoring that accompanies prolonged aminoglycoside administration is something that patients and clinicians should not have to contend with in the 21st century. Alternative therapies need to be defined for these infections, as there appear to be safer and more convenient alternatives available that await validation in larger clinical studies. This patient demonstrated bacteremia clearance and had a successful clinical outcome with daptomycin plus ceftaroline along with appropriately timed valve replacement surgery. The fact that the valvular tissue was still culture positive despite 2 weeks of therapy underscores the importance of surgical intervention in these cases, and it is unknown if medical therapy alone would have sufficed in this case, particularly with potential relapse after a regimen of ampicillin plus ceftriaxone that provided comparable killing *in vitro*.

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