Treatment of High-Level Gentamicin-Resistant

*Enterococcus faecalis* Endocarditis with Daptomycin Plus

Ceftaroline

George Sakoulas(1), Poochit Nonejuie(2), Victor Nizet(1), Joseph Pogliano(2), Nancy
Crum-Cianflone(3) and Fadi Haddad(4)

1. Department of Pediatric Pharmacology and Drug Discovery, and
2. Department of Biology
University of California San Diego School of Medicine, La Jolla, CA
3. Division of Infectious Diseases, Naval Medical Center San Diego, San Diego, CA
4. Sharp Grossmont Hospital, La Mesa, CA

Address Correspondence to:
George Sakoulas, MD
University of California San Diego School of Medicine
Department of Pediatrics-MC0687
9500 Gilman Drive
La Jolla, CA 92037-0687
Email: gsakoulas@ucsd.edu
Phone: 858-534-2325
Fax: 858-534-5611

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Abstract

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 in 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 past medical history significant for hypertension presented with 1 month of fevers. The patient received levofloxacin and doxycycline for presumed prostatitis. Physical examination revealed a grade 2 systolic murmur and grade 1 diastolic murmur. Blood cultures obtained were positive for Enterococcus faecalis. The patient was admitted to the hospital and started on ampicillin-sulbactam and gentamicin. White blood cell count (WBC) was 10,100 cells/mm³, hemoglobin 14 g/dL and 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 non-coronary cusp of the aortic valve. On the third hospital day gentamicin was discontinued and ceftriaxone 1 g IV q 12 h was started accompanying ampicillin 2 g IV q 4 h. 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 12 g continuous infusion over 24 h and ceftriaxone 1 g IV q 12 h were started initially. On hospital day 2, ceftriaxone was switched to daptomycin 8 mg/kg IV daily given 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 hours of daptomycin plus ampicillin therapy turned positive for same isolate after 4 and 3 days respectively.

Based on unpublished *in vitro* observations in our laboratory demonstrating synergy between daptomycin and ceftaroline against several clinical bloodstream isolates of *E. faecalis* and *E. faecium*, and a published report of synergy between daptomycin and ceftaroline against MRSA, ampicillin was discontinued and ceftaroline 600 mg IV every 8 h was added to daptomycin, with successful clearance of the bacteremia. The patient was discharged on daptomycin 8 mg/kg IV daily and ceftaroline 600 mg IV every 8 h and 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.

Based on this excellent clinical and microbiological response, we performed checkerboard assays and kill curves at clinically relevant antibiotic concentrations in Mueller Hinton broth supplemented to 50 mg/L 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/L, respectively. The organism was qualitatively negative for beta-lactamase production by nitrocefin disk. Checkerboard showed 4-fold reduction in daptomycin MIC in ceftaroline 0.5-16 mg/L and ampicillin 8 mg/L (Table). No differences in MIC
were observed in synergy studies between ampicillin and ceftaroline or ampicillin and ceftriaxone.

Kill curve assays with daptomycin 2 mg/L plus ceftaroline 1 or 5 mg/L confirmed synergy, as had been observed in prior data with other clinical isolates that prompted selection of this combination for this patient (Figure 1A). In order to provide a context of this degree of killing with this combination compared to other regimens clinicians may consider, we performed similar assays to determine relative synergy of daptomycin and ampicillin (Figure 1B), ceftriaxone or ceftaroline with ampicillin (Figure 1C), and vancomycin and ceftaroline or (Figure 1D). These experiments showed i) bacteriostatic activity of vancomycin 15 mg/L and ampicillin 20 mg/L alone against this isolate as anticipated; ii) comparable synergy with ampicillin 20 mg/L and either ceftriaxone 20 mg/L or ceftaroline 1 mg/L; iii) lack of synergy of ceftaroline with vancomycin.

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

Also similar to what we had observed with *E. faecium*, 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 (Figure 3). Note that this strain was much more susceptible to ampicillin and ceftaroline than the previously
published *E. faecium*, and therefore much lower concentrations of drugs were used to allow experimental growth 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 see-saw effect across the enterococcal species, and a potential area of further study of the differences in endovascular pathogenicity between *E. faecium* and *E. faecalis*.

Assessment of surface charge with or without ceftaroline or ampicillin using cytochrome c binding assays showed no significant differences in this property (data not shown), 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 when using 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 recommended in the literature and may have set up treatment failure. While limited to a single case, these results point to several alternative avenues of therapy that need to be studied clinically in 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, ampicillin and gentamicin appear straightforward on treatment guidelines. However in the practical clinical world, when not limited by HLGR as in this case, the otovestibular toxicity, nephrotoxicity, and therapeutic drug monitoring ‘baggage’ 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 but awaiting 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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Conflicts of Interest

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References


Table. Reduction of daptomycin (DAP) MIC in Mueller-Hinton broth supplemented to Ca$^{2+}$ 50 mg/L containing incrementally higher concentrations of ceftaroline (CPT) or ampicillin (AMP).

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Figure 1

A

B

C

D
Figure 1. Time kill assays (24 hr) in Mueller Hinton broth supplemented to Ca$^{2+}$ 50 mg/L evaluating the activity of daptomycin (DAP) alone or with ceftaroline (CPT) [A] or ampicillin (AMP) [B] against *E. faecalis*. Similar experiments showing effect of AMP with either ceftriaxone (CRO) or CPT [C] and vancomycin (VAN) with CPT [D] against *E. faecalis*. Mean of 3 experiments are shown, with duplicate plating for each, limit of detection log$_{10}$CFU/mL=3.0.
Figure 2. *E. faecalis* labeled with Bodipy-Daptomycin 16 mg/L (4X MIC, baseline MIC 4 mg/L) in LB broth for 15 min after 45 min-treatment with either AMP 10 mg/L, CPT 1 mg/L or 5 mg/L compared to control untreated cells. Normalized total intensity of signal represented per cell (bottom left) and number of binding spots/cell (bottom right). Microscopy method details are described in references 2 and 8.
Figure 3. Percent survival after 2 hours of *E. faecalis* in 64 µM (A) and 128 µM (B) cathelicidin LL37 comparing untreated controls to cells grown overnight in either AMP 4 mg/L or CPT 0.1 mg/L. Method details are provided in reference 2.