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Ampicillin in Combination with Ceftaroline, Cefepime, or Ceftriaxone Demonstrates Equivalent Activities in a High-Inoculum *Enterococcus faecalis* Infection Model

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Ampicillin-ceftriaxone combination therapy has become a predominant treatment for serious *Enterococcus faecalis* infections, such as endocarditis. Unfortunately, ceftriaxone use is associated with future vancomycin-resistant enterococcus colonization. We evaluated *E. faecalis* in an *in vitro* pharmacodynamic model against simulated human concentration-time profiles of ampicillin plus ceftaroline, cefepime, ceftriaxone, or gentamicin. Ampicillin-cefepime and ampicillin-ceftaroline demonstrated activities similar to those of ampicillin-ceftriaxone against *E. faecalis*.

Enterococcus faecalis is one of the most common causes of infective endocarditis in hospitalized and/or immunocompromised patients. The combination regimen of ampicillin plus ceftriaxone has averted high-level aminoglycoside resistance (HLAR) and improved the safety profile in *E. faecalis* endocarditis treatment over the traditional regimen of ampicillin plus gentamicin (1–4). Accordingly, ampicillin and ceftriaxone were recently added as an option to treat both HLAR and non-HLAR *E. faecalis* endocarditis, according to national guidelines (5). While this regimen has increased safety for patients with serious *E. faecalis* infections, it may create long-term collateral damage, as ceftriaxone carries an increased risk of vancomycin-resistant *Enterococcus* (VRE) gastrointestinal colonization (6, 7). This increase in VRE colonization is likely due to ceftriaxone's high biliary excretion and is associated with increased risk for VRE bacteremia (6–9). Cefepime and ceftaroline are cephalosporins with different spectra of activity, distinct structures, and less biliary excretion; therefore, they should carry less risk of VRE colonization (6, 10). We therefore evaluated the activities of these dual β-lactam combinations that have less potential for VRE colonization in a high-inoculum *in vitro* pharmacodynamic (IVPD) infection model (6, 10, 11).

(A portion of the results were presented as a poster at the 54th Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], 8 September 2014, Washington, DC.)

We evaluated two previously described strains of *E. faecalis*: one ampicillin-susceptible and gentamicin-susceptible strain (OG1X), and a β-lactamase-producing although still ampicillin-susceptible and HLAR gentamicin-resistant strain (HH22) (12, 13). Mueller-Hinton broth (MHB; Becton Dickinson, Sparks, MD, USA), adjusted to 25 mg/liter calcium and 12.5 mg/liter magnesium, was used for *in vitro* pharmacodynamic models (14). Colony counts were determined using tryptic soy agar (TSA; Difco, Becton Dickinson). Ampicillin (App Pharmaceuticals, Schaumberg, IL), ceftriaxone (Hospira, Lake Forest, IL), cefepime (Sagent Pharmaceuticals, Schaumberg, IL), ceftaroline research powder (Forest Laboratories, New York, NY), and gentamicin (Sigma-Aldrich, St. Louis, MO) were evaluated.

A previously described IVPD model was used to evaluate several antibiotic regimens against enterococci at a high inoculum ($\sim 10^8$ CFU/ml) over 24 h (15, 16). A 250-ml working-volume glass model with inflow and outflow ports controlled by peristaltic pumps was used to achieve desired antibiotic concentrations and half-lives. The regimens simulated free serum concentrations of human doses, including 2 g of ampicillin every 4 h (maximum concentration in serum [C_{max}], 150 μg/ml; protein binding, 20%; half-life, 1 h), 2 g of ceftriaxone every 12 h (C_{max}, 257 μg/ml; protein binding, 90%; half-life, 6 h), 2 g of cefepime every 12 h (C_{max}, 163.9 μg/ml; protein binding, 20%; half-life, 2 h), 600 mg of ceftaroline every 12 h (C_{max}, 21.3 μg/ml; protein binding, 20%; half-life, 2.66 h), and 6 mg/kg of body weight of gentamicin every 24 h (C_{max}, 24 μg/ml; protein binding, 0%; half-life, 2 h) (17–22). For combination regimens, the rate was set for the drug with the shorter half-life; the drug with the longer half-life was supplemented. The following regimens were tested: ampicillin alone, ceftriaxone alone, cefepime alone, ampicillin plus ceftriaxone, ampicillin plus cefepime, ampicillin plus ceftaroline, ampicillin plus gentamicin, and no antibiotic (growth control).

All model experiments were performed at least in duplicate to ensure reproducibility. Samples were removed from each model at 0, 4, 8, and 24 h. Once removed, samples were serially diluted, plated on TSA, and incubated at 37°C for 24 h before colony count enumeration. The limit of detection for this method is 2.0 $\log_{10}$ CFU/ml. Antimicrobial carryover was minimized by serial dilution (1:10 to 1:10,000) of plated samples in conjunction with vacuum filtration, if needed, as previously described (23).

The MICs of the antimicrobial agents were determined by Etest methodology (Table 1). All samples were incubated at 37°C in

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TABLE 1 MICs by Etest against two strains of *E. faecalis*

Drug	MIC (mg/liter) (CLSI susceptibility)[a]	
	OG1X, aminoglycoside susceptible	HH22, high-level aminoglycoside resistant
Ampicillin	0.19 (S)	2 (S)
Ceftriaxone	>256*	>256*
Cefepime	3*	16*
Ceftaroline	0.047*	0.5*
Gentamicin	12 (S)	>500 (R)

[a] S, susceptible per CLSI guidelines; R, resistant per CLSI guidelines. *, no CLSI breakpoints for cephalosporins against enterococci.

ambient air for 24 h. Etests were also used to assess changes in MIC at 24 h to detect resistance.

Changes in $\log_{10}$ CFU/ml were plotted to demonstrate reduction by each regimen over 24 h. Bactericidal activity (99.9% kill) was defined as a $\geq$3-$\log_{10}$ CFU/ml reduction and bacteriostatic activity as a <3-$\log_{10}$ CFU/ml change in colony count from the initial inoculum.

Changes in bacterial growth ($\log_{10}$ CFU/ml) at 24 h were compared by analysis of variance with Tukey's *post hoc* test. A *P* value of <0.05 was considered significant. All statistical analyses were performed using SPSS statistical software (SPSS 22, Inc., Chicago, IL).

Against both isolates, ampicillin-cefepime and ampicillin-ceftaroline demonstrated greater activity than that of ampicillin-gentamicin at 24 h (mean difference in $\log_{10}$ CFU/ml, 2.29 to 3.69; $P \leq 0.02$ for all) (Fig. 1). The activity of ampicillin-ceftriaxone was not significantly different than that of ampicillin-ceftaroline or ampicillin-cefepime. Ampicillin-gentamicin was no more active than ampicillin alone against either strain, likely due to high-level aminoglycoside resistance of HH22 and once-daily gentamicin dosing.

Against the gentamicin-susceptible *E. faecalis* OG1X, ampicillin alone and all ampicillin-cephalosporin combinations demonstrated bactericidal activity. Due to the considerable activity of ampicillin alone, ampicillin-cephalosporin combinations were not significantly more active. Ampicillin-gentamicin demonstrated regrowth at 24 h.

We used a high-dose once-daily gentamicin regimen in this study, similar to that recommended by recent European guidelines for enterococcal endocarditis (24). Previous studies have found no difference in humans or rabbits with gentamicin intervals of once, twice, or thrice daily (25, 26). Of importance, penicillins can accelerate the degradation of aminoglycosides *in vitro* (41). It remains possible, however, that activity against the gentamicin-susceptible isolate could have been increased by using gentamicin every 8 or 12 h. Additionally, an increase in other antibiotics, such as ceftaroline every 8 h, might increase activity against enterococci, particularly those with higher ceftaroline MICs.

Against our high-level aminoglycoside-resistant (HLAR), β-lactamase-producing ampicillin-susceptible isolate (HH22), ampicillin alone demonstrated bacteriostatic activity, but combinations with cefepime or ceftaroline demonstrated bactericidal activity at 24 h. These ampicillin-cefepime and ampicillin-ceftaroline combinations were more active than ampicillin alone (mean difference in $\log_{10}$ CFU/ml, 2.49; 95% confidence interval [95% CI], 0.46 to 4.52; $P = 0.01$, and 3.62; 95% CI, 1.59 to 5.65; $P = 0.001$). Ampicillin-ceftriaxone did not significantly increase activity over that of ampicillin alone but was also not significantly different from the other cephalosporin combinations. Against this HLAR isolate, there was regrowth at 24 h with all monotherapy regimens and ampicillin-gentamicin. Despite regrowth, no increases in MIC were seen with any combination or ampicillin monotherapy. There was no regrowth with ampicillin-cefepime or ampicillin-ceftaroline.

Enterococcal β-lactamase production has a negligible effect on ampicillin MIC at standard testing inocula but raises the MIC when tested at high inocula (27). The significant differences we observed in the activities of ampicillin alone against the two strains in this study are consistent with these prior studies of HH22. Our results are encouraging in that the reduced efficacy of ampicillin against the β-lactamase-producing strain was overcome by the addition of cefepime or ceftaroline. Our study is limited by the use of two strains and the 24-h duration. Additional research will be required before the efficacy of cefepime and ceftaroline against *E. faecalis* endocarditis in the clinical setting can be determined.

Overall, ampicillin-cephalosporin combinations demonstrated the greatest activity against both strains. Activity with dual-β-lactam therapy may be isolate dependent and likely depends on the isolate's susceptibility to ampicillin alone. While the rate of susceptibility to ampicillin remains at >95% in *E. faecalis*, up to 60% of bloodstream isolates in the United States in 2010 were gentamicin resistant, necessitating other synergistic treatment options (28–30). The synergy of ampicillin-cephalosporin combinations is thought to be due to complementary penicillin binding protein (PBP) saturation (31). Cephalosporins bind to PBP 2 and 3 at low concentrations, providing total saturation. Ampicillin binds to PBP 4 and 5, inhibiting cell wall synthesis (31). This same mechanism has been shown with amoxicillin-cefotaxime and amoxicillin-ceftriaxone (31, 32). The synergistic effect demonstrated in our study between ampicillin-cefepime and ampicillin-ceftaroline is predictable and increases the possibilities of additional treatment options for enterococcal endocarditis.

Previous studies have associated ceftriaxone use with both VRE colonization and bacteremia (7, 8). The high biliary excretion of ceftriaxone selects for the survival of VRE; this increased colonization does not occur with other cephalosporins that do not undergo significant biliary excretion (6, 7, 9, 11). The high levels of ceftriaxone in the gastrointestinal tract (up to 67% biliary excretion) inhibit colonic microbiota, but due to their intrinsic resistance to cephalosporins (along with ampicillin and vancomycin resistance), VRE growth is left unchecked (7, 33). The complex interactions between colonic flora, innate immunity, antimicrobial spectrum, and gastrointestinal antimicrobial concentration likely all contribute to VRE colonization, but these relationships have not been clearly determined (7, 33). Antienterococcal and antianaerobic activities are important for VRE colonization, but VRE expansion does not always correlate with the numbers of anaerobes present (10, 34). Both gut anaerobes and Gram-negative bacteria interact with VRE growth and the immune regulation of VRE (34, 35). In hospitalized patients, rates of VRE acquisition can be as high as 41% (30). Bacteremia with HLAR enterococci, which has demonstrated increased mortality over that with non-HLAR bacteremia, was also associated with previous third-generation cephalosporin use, likely ceftriaxone (36).

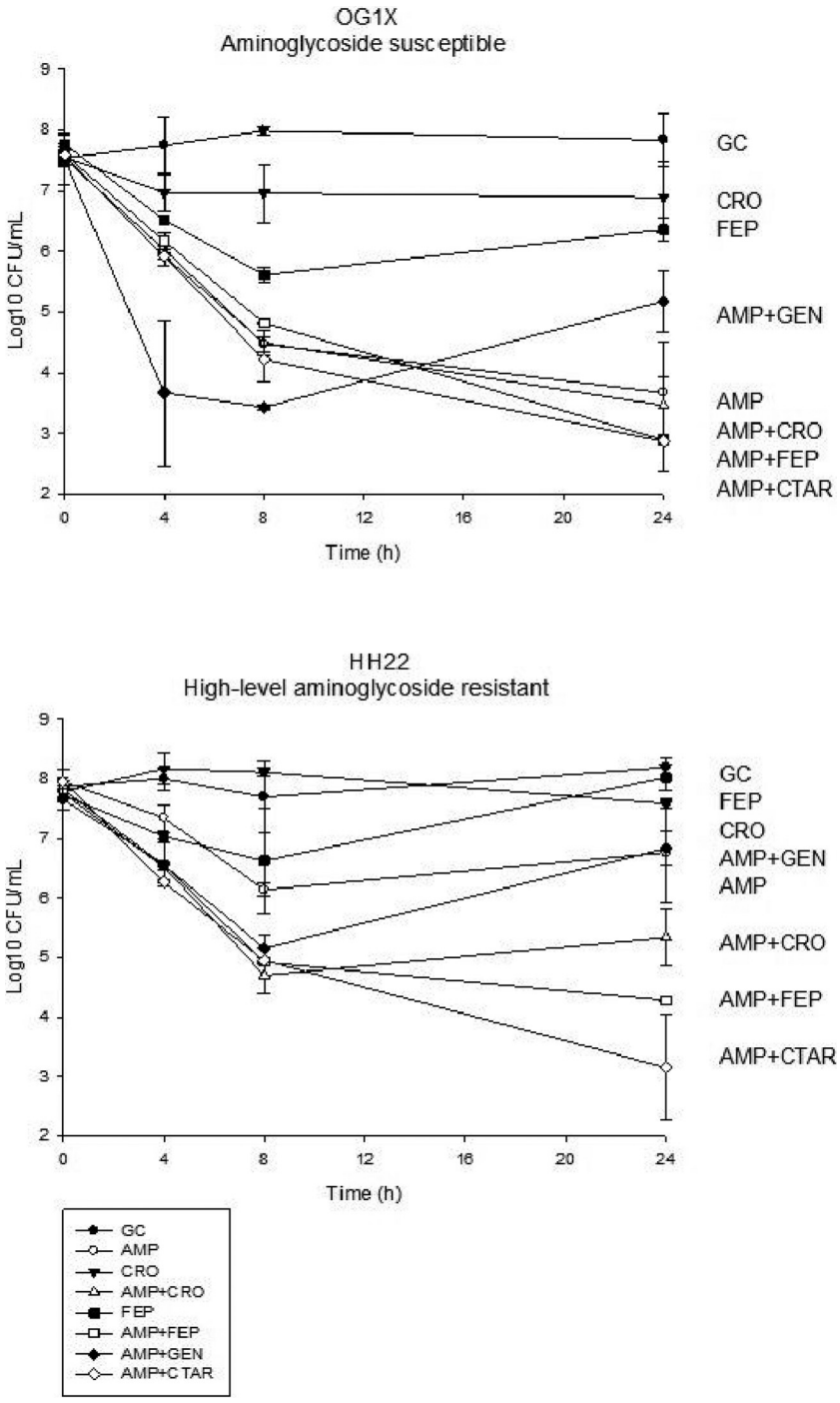

FIG 1 Activities (mean $\log_{10}$ CFU/ml ± standard deviation) of ampicillin (AMP), ceftriaxone (CRO), cefepime (FEP), ceftaroline (CTAR), gentamicin (GEN), and growth control (GC) regimens against two *E. faecalis* isolates over 24 h in an *in vitro* pharmacodynamic model.

Cefepime carries less risk of promoting VRE colonization in animals and has not been associated with VRE in humans (6, 10). This may be due to the low biliary excretion (~95% renal excretion) and lack of antianaerobic activity (10, 19). The narrower spectrum of activity of ceftaroline, coupled with its renal excretion and high activity in this study, make ampicillin-ceftaroline a promising combination for *E. faecalis* endocarditis. Despite intrinsic resistance, ceftaroline monotherapy has demonstrated *in vitro* and *in vivo* activity against *E. faecalis* (37). The ampicillin-ceftaroline combination has previously demonstrated synergy against *E. faecalis* in an *in vitro* time-kill study (38). Further study of VRE colonization with ceftaroline is needed, as the antianaerobic activity is ~4- to 8-fold greater than that of ceftriaxone, but biliary excretion is lower (~6% excreted in feces) (39, 40).

In our study, ampicillin-cephalosporin combinations demonstrated the most activity against both strains of *E. faecalis* over 24

h. Ampicillin-cefepime and ampicillin-ceftaroline significantly increased activity over that of ampicillin alone for one strain. Dual-β-lactam regimens should be investigated further, not only for activity, but also with regard to colonization and infection with vancomycin-resistant enterococci.

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