AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EXPERIMENTAL THERAPEUTICS

Pharmacodynamics of Ceftaroline plus Ampicillin against *Enterococcus faecalis* in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model of Simulated Endocardial Vegetations

Brian J. Werth, Laura M. Shireman

Department of Pharmacy University of Washington School of Pharmacy, Seattle, Washington, USA

ABSTRACT The combination of ampicillin plus ceftaroline has been suggested to be more reliably synergistic against *Enterococcus faecalis* than ampicillin plus ceftriaxone using time-kill methods. The purpose of this study was to determine if this trend persists in a two-compartment model of simulated endocardial vegetations (SEV) using clinically relevant pharmacokinetic exposures of these antimicrobials. Three clinically derived *E. faecalis* strains were included in the study. The MICs of study antimicrobials were determined by broth microdilution. Simulations of ampicillin (2 g every 4 h [q4h]; maximum concentration of drug in serum [C_{max}], 72.4 mg/liter; half-life [$t_{1/2}$], 1.9 h), ceftaroline-fosamil (600 mg q8h; C_{max}, 21.3 mg/liter; $t_{1/2}$, 2.66 h), ceftriaxone (C_{max}, 257 mg/liter; $t_{1/2}$, 8 h), and ampicillin plus ceftaroline and ampicillin plus ceftriaxone were evaluated against 3 strains of *E. faecalis* isolated from patients with endocarditis in an *in vitro* PK/PD SEV model over 72 h, with a starting inoculum of ~9 $\log_{10}$ CFU/g. All strains were susceptible to ampicillin (MIC, ≤2 mg/liter). Ceftaroline MICs varied from 2 to 16 mg/liter. All strains had ceftriaxone MICs of 256 mg/liter. W04 and W151 exhibited high-level aminoglycoside resistance but W07 did not. Ampicillin plus ceftaroline resulted in significantly greater reductions in CFU per gram by 72 h than ampicillin for all strains ($P \leq 0.025$) than ampicillin plus ceftriaxone for W04 ($P = 0.019$) but not W07 or W151 ($P \geq 0.15$). A 4-fold increase in ampicillin MIC was observed for W07 at 72 h, but this was prevented by the addition of ceftaroline or ceftriaxone. The combination of ampicillin plus ceftaroline appears to be at least as efficacious as ampicillin plus ceftriaxone and may lead to improved activity against some strains of *E. faecalis*, but these differences may be small and the clinical significance should not be overestimated.

KEYWORDS ceftriaxone, endocarditis, HLAR, PK/PD, dual β-lactam, infective endocarditis, synergy

Received 18 October 2016 **Returned for modification** 3 December 2016 **Accepted** 12 January 2017

Accepted manuscript posted online 17 January 2017

Citation Werth BJ, Shireman LM. 2017. Pharmacodynamics of ceftaroline plus ampicillin against *Enterococcus faecalis* in an *in vitro* pharmacokinetic/pharmacodynamic model of simulated endocardial vegetations. Antimicrob Agents Chemother 61:e02235-16. https://doi.org/10.1128/AAC.02235-16.

Address correspondence to Brian J. Werth, bwerth@uw.edu.

Enterococci are an increasingly prevalent cause of infective endocarditis, especially hospital-acquired endocarditis (1). The overwhelming majority (≥90%) of enterococcal endocarditis infections can be attributed to *Enterococcus faecalis* (2). Despite the fact that in most locales *E. faecalis* remains broadly susceptible to many antimicrobials, including ampicillin, clinical outcomes of enterococcal endocarditis remain poor (2, 3). The combination of ampicillin plus ceftriaxone for the treatment of *E. faecalis* infective endocarditis appears to be as effective as but less toxic than the traditional combination of ampicillin plus gentamicin (4). Accordingly, the 2015 infective endocarditis practice guidelines endorse ampicillin plus ceftriaxone as a reasonable choice not just in the setting of high-level aminoglycoside resistance but also for patients who may not be suitable candidates for aminoglycoside therapy (5). Nevertheless, given the

TABLE 1 MICs of study antimicrobials and vancomycin

Strain	MIC (μg/ml)				
	Ampicillin	Ceftaroline	Ceftriaxone	Vancomycin	HLGR[a]
W04	0.5	8	256	1	+
W07	2	16	256	1	−
W151	1	2	256	2	+

[a]HLGR, high-level gentamicin resistance.

poor outcomes of enterococcal endocarditis, it is easy to argue that more efficacious therapies are needed.

Synergy between ampicillin and ceftriaxone is mediated through differential penicillin binding protein (PBP) affinities between these two drugs, which leads to greater saturation of more PBP subtypes, thereby maximally inhibiting cell wall synthesis (6). Ceftaroline has affinity for a broad range of Gram-positive PBPs, including PBPs 2 and 3, which are targeted by 3rd-generation cephalosporins but also PBP5 of enterococci, which can be overexpressed in strains with reduced susceptibility to ampicillin (7–9). Consequently, we recently investigated the combination of ampicillin plus ceftaroline, which has this theoretical pharmacologic advantage over the combination of ampicillin plus ceftriaxone, in a time-kill synergy study (10). In these experiments, ampicillin plus ceftaroline was synergistic against all 7 *E. faecalis* strains tested and resulted in significantly lower CFU per milliliter at 24 h than ampicillin plus ceftriaxone against 4 of 7 strains tested. Given these promising preliminary findings, it was important to further assess these combinations in more complex models simulating human pharmacokinetic exposures and the tissue barrier to drug penetration presented by endocardial vegetations. As such, the purpose of this study was to determine if the combination of ampicillin plus ceftaroline is more active *in vitro* than ampicillin plus ceftriaxone at clinically relevant, pharmacokinetic exposures using a well-validated pharmacokinetic/pharmacodynamic (PK/PD) model of simulated endocardial vegetations (SEV).

RESULTS

Susceptibility testing. MIC values for *E. faecalis* strains W04, W07, and W151 are listed in Table 1. All strains were susceptible to ampicillin and vancomycin. There are no established susceptibility criteria for cephalosporins against enterococci. Strains W04 and W151 were highly gentamicin-resistant strains, while W07 was negative for high-level aminoglycoside resistance.

Pharmacodynamics. Pharmacodynamic responses to simulated antimicrobial regimens for each strain are summarized in Fig. 1, and changes in CFU per gram over 72 h are detailed in Table 2. Ampicillin monotherapy was bactericidal against W04 and W07 but was bacteriostatic against W151. Both combinations were bactericidal against all strains at 72 h. The combination of ampicillin plus ceftaroline demonstrated significantly improved killing compared to ampicillin alone against all strains ($P \leq 0.025$). The combination of ampicillin plus ceftaroline was significantly more active than ampicillin plus ceftriaxone against W04 ($P = 0.019$) but was similarly efficacious against W07 or W151 ($P \geq 0.15$). Ampicillin plus ceftriaxone was significantly more active than ampicillin alone against W151 and W04 ($P \leq 0.034$) but did not significantly improve killing against W07 ($P = 0.27$). Both combinations met criteria for therapeutic enhancement against W151 but not for W04 or W07.

Pharmacokinetics. Achieved PK parameters for ampicillin were a maximum concentration of drug in serum (C_{max}) of 67.38 mg/liter (95% confidence interval [CI], 65.3 to 69.4 mg/liter) and a half-life ($t_{1/2}$) of 1.96 h (95% CI, 1.55 to 2.65 h). The average time above the MIC for ampicillin was 100% for all strains. Achieved PK parameters for ceftaroline were a C_{max} of 20.35 mg/liter (95% CI, 19.78 to 20.92 mg/liter) and $t_{1/2}$ of 2.54 h (95% CI, 2.15 to 3.11 h). Achieved PK parameters for ceftriaxone were a C_{max} of 219.5 mg/liter (95% CI, 216.3 to 222.8 mg/liter) and $t_{1/2}$ of 7.6 h (95% CI, 5.2 to 13.9 h). These achieved PK parameters are summarized in Fig. 2.

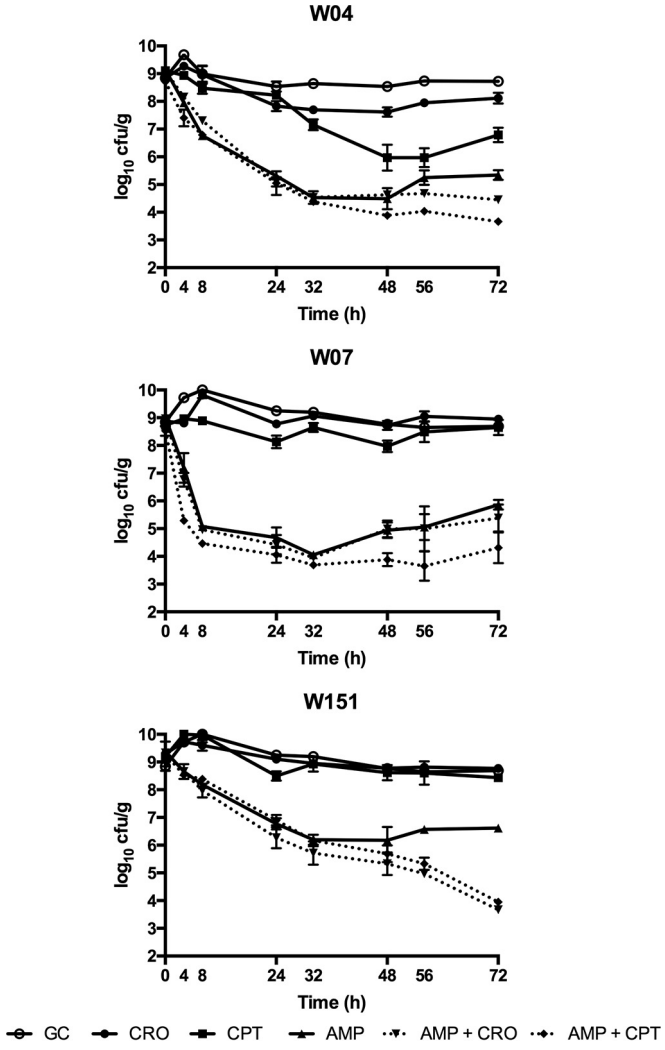

FIG 1 Pharmacodynamics of simulated antimicrobial regimens. GC, growth control; CRO, 2 g ceftriaxone intravenously every 12 h; CPT, 600 mg ceftaroline fosamil intravenously every 8 h; AMP, 2 g ampicillin intravenously every 4 h; AMP+CRO, ampicillin plus ceftriaxone; AMP+CPT, ampicillin plus ceftaroline.

Changes in susceptibility. Isolates with ampicillin MICs higher than the baseline were not detected on resistance screening plates for W04 and W151. Ampicillin monotherapy selected for a W07 derivative with an ampicillin MIC of 8 μg/ml, up from 2 μg/ml at baseline (susceptibility breakpoint, $\leq$8 μg/ml). These less susceptible clones were detected at a rate of 0.0076% of surviving colonies at the 72-h time point in both models. Significant changes in susceptibility were not detected at earlier time points.

TABLE 2 Change in CFU over 72 h

Regimen[a]	Change in $\log_{10}$ CFU/g from 0 to 72 h for strain[b]:		
	W04	W07	W151
CRO	−0.72	0.08	−0.52
CPT	−2.31	−0.08	−0.75
AMP	−3.8	−3.15	−2.76
AMP + CRO	−4.65*	−3.58	−5.66*
AMP + CPT	−5.05*,§	−4.16*	−5.34*

[a]CRO, simulations of 2 g ceftriaxone intravenously every 12 h; CPT, 600 mg ceftaroline intravenously every 8 h; AMP, 2 g ampicillin intravenously every 4 h.
[b]Symbols: *, significantly greater $\log_{10}$ CFU/g reduction than with AMP ($P \leq 0.05$); §, significantly greater $\log_{10}$ CFU/g reduction than with AMP + CRO alone ($P \leq 0.05$).

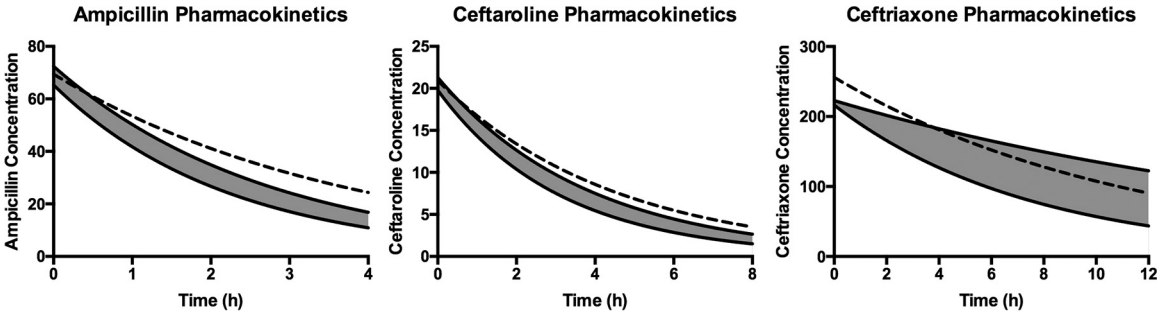

FIG 2 Dashed lines indicate the pharmacokinetic curves targeted in the model. The shaded areas indicate the bounds of the 95% confidence interval of the measured drug concentrations for all of the models across the dosing interval for each drug.

The addition of ceftriaxone or ceftaroline to ampicillin prevented meaningful changes in the ampicillin MIC.

DISCUSSION

The results of this study illustrate the challenge we face in optimizing antibacterial activity against *E. faecalis* in the setting of endocarditis. Despite robust synergistic effects observed for the combination of ampicillin plus ceftaroline relative to the combination of ampicillin plus ceftriaxone in the initial time-kill study, the present PK/PD study in an SEV model shows a relatively small improvement of either combination. Therapeutic enhancement, the PK/PD equivalent of synergy, was only achieved against one strain (W151). Overall, ampicillin plus ceftaroline was significantly more active than ampicillin plus ceftriaxone against one strain, numerically improved against another, and not significantly or appreciably different against the last strain. This seems to suggest that this combination is at least as active as ampicillin plus ceftriaxone against most strains but may be more active against some. It remains to be seen whether it will be possible to identify strains that have improved response to ampicillin plus ceftaroline compared to ampicillin plus ceftriaxone.

Contrary to the standard dogma, ampicillin monotherapy actually achieved bactericidal activity against W04 and W07 by 24 h, which persisted through 72 h, but not against W151. This was observed despite the fact that we simulated ampicillin exposures on the lower end of what is reported in the literature, which is surprisingly variable across different studies (11–13). We chose to simulate lower exposures in our model in an attempt to increase the chances of observing a difference in the combination regimens. However, it is important to note that the time above the MIC ($T>$MIC), which is the PK/PD parameter that predicts efficacy of beta-lactams, was 100% of the dosing interval in all models. In fact, the free drug concentration at the end of the dosing interval (fC_{min}) was approximately 13 μg/ml (assuming 20% protein binding) on average, or 6.5 times the MIC of the least susceptible organism, W07. While it is commonly held that ampicillin is bacteriostatic against enterococci, other *in vitro* studies have observed bactericidal activity of ampicillin monotherapy (13–15).

Like other studies with a similar design, this study has some limitations. Infective endocarditis is a disease state that is treated for an extended period of time, typically for about 6 weeks. It is possible that if we continued the model for longer we would observe greater differences in activity that might illustrate more clearly whether there is an advantage of one combination over the other. A similar study evaluating the activity of ampicillin in combination with ceftaroline, cefepime, and ceftriaxone failed to detect any difference in the efficacy of these combinations (13). However, in that study the model was only run for 24 h, which may not be long enough to observe differences in activity. Indeed, in our present study, there was no difference in activity between ampicillin or either of the combinations at 24 h, and the differences between ampicillin monotherapy and combination regimens did not begin to emerge until after 48 h. Changes in ampicillin susceptibility were only just beginning to occur in the least susceptible strain at 72 h, so it is possible that if these subpopulations were enriched under continued

ampicillin monotherapy, the combinations would have improved activity further compared to ampicillin alone, making these differences in activity more apparent.

Given the poor clinical outcomes associated with enterococcal endocarditis and the limited pharmacodynamic response of the standard therapies, future work is necessary to innovate highly bactericidal antimicrobial regimens. High-dose daptomycin-based combinations look promising but have been studied mostly in the context of ampicillin- and vancomycin-resistant enterococci or as salvage therapy (16–22). Further exploration into these combinations, such as daptomycin plus ampicillin or daptomycin plus ceftaroline, against ampicillin-susceptible *E. faecalis*, which causes the majority of invasive enterococcal infections, may be valuable.

Based on the currently available literature and the results of this study, the combination of ampicillin plus ceftaroline appears to be at least as active as ampicillin plus ceftriaxone against *E. faecalis* derived from patients with infective endocarditis and may lead to improved activity against some strains. However, these differences may be small, and the clinical significance should not be overestimated. Furthermore, these potential benefits may be limited by institutional antimicrobial stewardship considerations that may restrict ceftaroline use in this setting.

MATERIALS AND METHODS

Bacterial strains. Three strains of ampicillin-susceptible *Enterococcus faecalis* isolated from the blood of patients with a confirmed diagnosis of infective endocarditis were evaluated in the model. All strains were confirmed as *E. faecalis* by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) prior to the experiments.

Antimicrobials and media. All *in vitro* experiments were performed in Mueller-Hinton II broth (MHB). Colony counts and subculture of enterococci were performed on brain heart infusion agar (BHIA). Resistance screening was performed on BHIA supplemented with ampicillin. Ampicillin, vancomycin, gentamicin, and ceftriaxone were purchased from commercial sources. *In vitro*-active ceftaroline dihydrochloride was provided by its manufacturer (Allergan, Dublin, Ireland).

Susceptibility testing. MIC values of study antimicrobials were determined in duplicate by manual broth microdilution at approximately 10^6 CFU/ml according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (23). High-level gentamicin resistance was screened by broth microdilution in accordance with CLSI guidelines.

SEVs. SEVs were prepared by mixing 0.05 ml of organism suspension (final inoculum, ~10^9 CFU/g), 0.5 ml of pooled human cryoprecipitated antihemophilic factor derived from at least 30 volunteer donors (Puget Sound Blood Center, Seattle, WA), and 0.025 ml of platelet suspension (platelets mixed with normal saline, 250,000 to 500,000 platelets per clot) in 1.5-ml microcentrifuge tubes. Recombinant human thrombin (625 U/ml), 0.05 ml, was added to each tube after insertion of a sterile monofilament line into the mixture to initiate clotting. After 45 min of incubation at room temperature, the resultant simulated vegetations were removed from the tubes with a sterile plastic needle and introduced into the model aseptically. This methodology results in SEVs consisting of approximately 3 to 3.5 g/dl of albumin and 6.8 to 7.4 g/dl of total protein (16, 24–26).

***In vitro* pharmacodynamic infection model.** A custom glass *in vitro* infection model with 2 ports for adding and removing medium, 1 port for dosing the antimicrobials, and 4 ports for SEV sampling was used in all of the experiments. The model was prefilled with 250 ml of MHB, and SEVs were introduced through the sampling ports and suspended in the medium. The model apparatus was maintained in a 37°C water bath throughout the procedure, and a magnetic stir bar was used to ensure mixing of the drug in the model. Antimicrobials were administered as repeated boluses over a 72-h period into the central compartment via an injection port. Fresh medium was continuously supplied and removed from the model along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL) set to simulate the half-lives of the antibiotics. Supplemental ceftaroline and ceftriaxone were added to combination models with ampicillin via a supplemental chamber to account for excessive clearance of these drugs by the higher flow rates required to simulate ampicillin elimination as previously described (27). The following drug exposures were simulated in the model: ampicillin at 2 g every 4 h (peak, 72.4 mg/liter; average $t_{1/2}$, 1.9 h), ceftaroline-fosamil at 600 mg every 8 h (peak, 21.3 mg/liter; average $t_{1/2}$, 2.66 h), ceftriaxone simulations of 2 g every 12 h (peak, 257 mg/liter; average $t_{1/2}$, 8 h), ampicillin plus ceftaroline, and ampicillin plus ceftriaxone (11, 28, 29). Drug-free growth controls were performed for each isolate for 3 days using the pump settings used to simulate ceftriaxone clearance to reduce waste.

Pharmacodynamic analysis. Two SEVs were removed from each model (total of 32) at 0, 4, 8, 24, 32, 48, 56, and 72 h for days 1 to 3. The SEVs were homogenized and diluted in cold saline and drop-plated onto BHIA plates. Plates were then incubated at 37°C for 24 h before colony enumeration. The change in $\log_{10}$ CFU/gram over 72 h was plotted against time to generate curves based on the number of remaining organisms over time. Bactericidal activity (99.9% kill) and bacteriostatic activity were defined as a $\geq$3-$\log_{10}$ CFU/ml or a $<$3-$\log_{10}$ CFU/ml reduction in colony count from the initial inoculum, respectively. The effects of the antimicrobial combinations were interpreted as follows. Therapeutic enhancement was defined as an increase in kill of $\geq$2 $\log_{10}$ CFU/g by a combination of antimicrobials

versus the most active single agent of that combination at 72 h. Improvement was defined as a 1- to 2-$\log_{10}$ CFU/g increase in kill compared to the most active single agent, while combinations that resulted in $\geq$1 $\log_{10}$ CFU/g of bacterial growth compared to the most-active single agent was considered antagonism.

Pharmacokinetic analysis. Pharmacokinetic samples were obtained through the injection port of each model at various time points for verification of target antibiotic concentrations. All samples were stored at −80°C until they were ready for analysis. Ampicillin, ceftriaxone, and ceftaroline concentrations were assayed using reverse-phase high-performance liquid chromatography with mass spectrometry detection (LC-MS) at the University of Washington School of Pharmacy analytical pharmacokinetic laboratory. The respective internal standards (IS) for each antibiotic were the following: ampicillin (IS, 400 ng of d_5-ampicillin), ceftaroline (IS, 1 μg cefaclor), and ceftriaxone (IS, 2 μg cefixime). Broth samples (25 μl) with the internal standards were added to 800 μl methanol and 800 μl 0.1% formic acid, vortexed, and subsequently centrifuged. The supernatant was transferred to a 96-well plate for LC-MS analysis. Samples were analyzed on an Agilent G1956B single-quadrupole mass spectrometer coupled to an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA) operating in electrospray positive ionization mode. Ions monitored were 350.1 (ampicillin), 355.1 (d_5-ampicillin), 368.0 (cefaclor), 454.0 (cefixime), 555.0 (ceftriaxone), and 605.1 (ceftaroline) *m/z*. Separation was achieved using an Agilent Zorbax SB-C_{18} column (2.1 mm by 150 mm by 5 μm; Agilent Technologies, Palo Alto, CA). The mobile phase consisted of 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B). The gradient began at 5% acetonitrile and was held at 5% until 1 min, increased to 40% acetonitrile by 4 min, increased to 50% acetonitrile by 6 min, increased to 90% acetonitrile by 6.1 min, was held at 90% acetonitrile until 9 min, and decreased to 5% acetonitrile by 9.5 min. The column was equilibrated for 3 min at 5% acetonitrile before the next injection.

An 8-point calibration curve was prepared by spiking blank broth with ampicillin (dynamic range, 1.38 to 138 μg/ml), ceftaroline (dynamic range, 0.344 to 34.4 μg/ml), or ceftriaxone (dynamic range, 3.4 to 340 μg/ml). The calibration curves were processed identically to the experimental samples. The ratio of peak heights of the antibiotics and their respective internal standards were analyzed by second-order polynomial regression. Pharmacokinetic analysis was performed in Prism 6 (GraphPad Software, Inc., San Diego, CA, USA) in order to calculate average half-lives, peak and trough concentrations, and time above the MIC (*T*>MIC) as appropriate for ampicillin.

Changes in susceptibility. At 72 h, samples of 0.1 ml were plated on BHIA containing 3 times the MIC of ampicillin for each strain to assess for meaningful changes (>1 $\log_2$ dilution) in ampicillin susceptibility. Plates were examined for growth after 24 and 48 h of incubation at 37°C. Broth microdilution MICs were performed on any isolate observed to grow on ampicillin screening plates. If resistance was detected by 72 h, then earlier time points were screened to detect the first occurrence of MIC elevation. Ceftaroline and ceftriaxone resistance was not assessed, since neither drug is indicated for enterococcal infections or has a susceptibility breakpoint.

Statistical analysis. In order to compare activity of each simulated regimen across the 72-h course, the differences in CFU/gram at 72 h and 0 h were calculated and compared by one-way analysis of variance with a Tukey post-hoc test for multiple comparisons or by multiple *t* tests as appropriate. A *P* value of $\leq$0.05 was considered significant. All calculations and statistical analyses were performed in Prism6.

ACKNOWLEDGMENTS

B.J.W. has received research support from Allergan and Merck. L.M.S. has no potential conflicts of interest.

This study was financially supported by an investigator-initiated grant from Allergan. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

REFERENCES

1. Nigo M, Munita JM, Arias CA, Murray BE. 2014. What's new in the treatment of enterococcal endocarditis? Curr Infect Dis Rep 16:431. https://doi.org/10.1007/s11908-014-0431-z.
2. Chirouze C, Athan E, Alla F, Chu VH, Ralph Corey G, Selton-Suty C, Erpelding ML, Miro JM, Olaison L, Hoen B, International Collaboration on Endocarditis Study Group. 2013. Enterococcal endocarditis in the beginning of the 21st century: analysis from the International Collaboration on Endocarditis-Prospective Cohort Study. Clin Microbiol Infect 19:1140–1147. https://doi.org/10.1111/1469-0691.12166.
3. Pericas JM, Cervera C, del Rio A, Moreno A, Garcia de la Maria C, Almela M, Falces C, Ninot S, Castaneda X, Armero Y, Soy D, Gatell JM, Marco F, Mestres CA, Miro JM, Hospital Clinic Endocarditis Study Group. 2014. Changes in the treatment of Enterococcus faecalis infective endocarditis in Spain in the last 15 years: from ampicillin plus gentamicin to ampicillin plus ceftriaxone. Clin Microbiol Infect Dis 20:O1075–O1083. https://doi.org/10.1111/1469-0691.12756.
4. Fernandez-Hidalgo N, Almirante B, Gavalda J, Gurgui M, Pena C, de Alarcon A, Ruiz J, Vilacosta I, Montejo M, Vallejo N, Lopez-Medrano F, Plata A, Lopez J, Hidalgo-Tenorio C, Galvez J, Saez C, Lomas JM, Falcone M, de la Torre J, Martinez-Lacasa X, Pahissa A. 2013. Ampicillin plus ceftriaxone is as effective as ampicillin plus gentamicin for treating enterococcus faecalis infective endocarditis. Clin Infect Dis 56:1261–1268. https://doi.org/10.1093/cid/cit052.
5. Baddour LM, Wilson WR, Bayer AS, Fowler VG, Jr, Tleyjeh IM, Rybak MJ, Barsic B, Lockhart PB, Gewitz MH, Levison ME, Bolger AF, Steckelberg JM, Baltimore RS, Fink AM, O'Gara P, Taubert KA, American Heart Association Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and Stroke Council. 2015. Infective endocarditis in adults: diagnosis, antimicrobial therapy, and management of complications: a scientific statement for healthcare professionals from the American Heart Association. Circulation 132:1435–1486. https://doi.org/10.1161/CIR.0000000000000296.

6. Mainardi JL, Gutmann L, Acar JF, Goldstein FW. 1995. Synergistic effect of amoxicillin and cefotaxime against Enterococcus faecalis. Antimicrob Agents Chemother 39:1984–1987. https://doi.org/10.1128/AAC.39.9.1984.
7. Rybkine T, Mainardi JL, Sougakoff W, Collatz E, Gutmann L. 1998. Penicillin-binding protein 5 sequence alterations in clinical isolates of Enterococcus faecium with different levels of beta-lactam resistance. J Infect Dis 178:159–163. https://doi.org/10.1086/515605.
8. Henry X, Verlaine O, Amoroso A, Coyette J, Frere JM, Joris B. 2013. Activity of ceftaroline against Enterococcus faecium PBP5. Antimicrob Agents Chemother 57:6358–6360. https://doi.org/10.1128/AAC.00923-13.
9. Kosowska-Shick K, McGhee PL, Appelbaum PC. 2010. Affinity of ceftaroline and other beta-lactams for penicillin-binding proteins from Staphylococcus aureus and Streptococcus pneumoniae. Antimicrob Agents Chemother 54:1670–1677. https://doi.org/10.1128/AAC.00019-10.
10. Werth BJ, Abbott AN. 2015. The combination of ampicillin plus ceftaroline is synergistic against Enterococcus faecalis. J Antimicrob Chemother 70:2414–2417. https://doi.org/10.1093/jac/dkv125.
11. Valcke YJ, Rosseel MT, Pauwels RA, Bogaert MG, Van der Straeten ME. 1990. Penetration of ampicillin and sulbactam in the lower airways during respiratory infections. Antimicrob Agents Chemother 34:958–962. https://doi.org/10.1128/AAC.34.6.958.
12. Meyers BR, Wilkinson P, Mendelson MH, Walsh S, Bournazos C, Hirschman SZ. 1991. Pharmacokinetics of ampicillin-sulbactam in healthy elderly and young volunteers. Antimicrob Agents Chemother 35:2098–2101. https://doi.org/10.1128/AAC.35.10.2098.
13. Luther MK, Rice LB, LaPlante KL. 2016. Ampicillin in combination with ceftaroline, cefepime, or ceftriaxone demonstrates equivalent activities in a high-inoculum Enterococcus faecalis infection model. Antimicrob Agents Chemother 60:3178–3182. https://doi.org/10.1128/AAC.03126-15.
14. Tripodi MF, Utili R, Rambaldi A, Locatelli A, Rosario P, Florio A, Ruggiero G. 1996. Unorthodox antibiotic combinations including ciprofloxacin against high-level gentamicin resistant enterococci. J Antimicrob Chemother 37:727–736. https://doi.org/10.1093/jac/37.4.727.
15. Lavoie SR, Wong ES, Coudron PE, Williams DS, Markowitz SM. 1993. Comparison of ampicillin-sulbactam with vancomycin for treatment of experimental endocarditis due to a beta-lactamase-producing, highly gentamicin-resistant isolate of Enterococcus faecalis. Antimicrob Agents Chemother 37:1447–1451. https://doi.org/10.1128/AAC.37.7.1447.
16. Hall Snyder A, Werth BJ, Barber KE, Sakoulas G, Rybak MJ. 2014. Evaluation of the novel combination of daptomycin plus ceftriaxone against vancomycin-resistant enterococci in an in vitro pharmacokinetic/pharmacodynamic simulated endocardial vegetation model. J Antimicrob Chemother 69:2148–2154. https://doi.org/10.1093/jac/dku113.
17. Sakoulas G, Bayer AS, Pogliano J, Tsuji BT, Yang SJ, Mishra NN, Nizet V, Yeaman MR, Moise PA. 2012. Ampicillin enhances daptomycin- and cationic host defense peptide-mediated killing of ampicillin- and vancomycin-resistant Enterococcus faecium. Antimicrob Agents Chemother 56:838–844. https://doi.org/10.1128/AAC.05551-11.
18. Sakoulas G, Nonejuie P, Nizet V, Pogliano J, Crum-Cianflone N, Haddad F. 2013. Treatment of high-level gentamicin-resistant Enterococcus faecalis endocarditis with daptomycin plus ceftaroline. Antimicrob Agents Chemother 57:4042–4045. https://doi.org/10.1128/AAC.02481-12.
19. Sakoulas G, Rose W, Nonejuie P, Olson J, Pogliano J, Humphries R, Nizet V. 2014. Ceftaroline restores daptomycin activity against daptomycin nonsusceptible vancomycin resistant Enterococcus faecium. Antimicrob Agents Chemother 58:1494–1500. https://doi.org/10.1128/AAC.02274-13.
20. Smith JR, Barber KE, Raut A, Aboutaleb M, Sakoulas G, Rybak MJ. 2015. β-Lactam combinations with daptomycin provide synergy against vancomycin-resistant Enterococcus faecalis and Enterococcus faecium. J Antimicrob Chemother 70:1738–1743. https://doi.org/10.1093/jac/dkv007.
21. Werth BJ, Barber KE, Tran KN, Nonejuie P, Sakoulas G, Pogliano J, Rybak MJ. 2015. Ceftobiprole and ampicillin increase daptomycin susceptibility of daptomycin-susceptible and -resistant VRE. J Antimicrob Chemother 70:489–493. https://doi.org/10.1093/jac/dku386.
22. Ramos MC, Grayson ML, Eliopoulos GM, Bayer AS. 1992. Comparison of daptomycin, vancomycin, and ampicillin-gentamicin for treatment of experimental endocarditis caused by penicillin-resistant enterococci. Antimicrob Agents Chemother 36:1864–1869. https://doi.org/10.1128/AAC.36.9.1864.
23. Clinical and Laboratory Standards Institute. 2015. M07-A10. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 10th ed. CLSI, Wayne, PA.
24. Werth BJ, Barber KE, Ireland CE, Rybak MJ. 2014. Evaluation of ceftaroline, vancomycin, daptomycin or ceftaroline plus daptomycin against daptomycin non-susceptible (DNS) methicillin resistant Staphylococcus aureus (MRSA) in an in vitro PK/PD model of simulated endocardial vegetations (SEV). Antimicrob Agents Chemother 58:3177–3181. https://doi.org/10.1128/AAC.00088-14.
25. Steed ME, Werth BJ, Ireland CE, Rybak MJ. 2012. Evaluation of the novel combination of high-dose daptomycin plus trimethoprim-sulfamethoxazole against daptomycin-nonsusceptible methicillin-resistant staphylococcus aureus using an in vitro pharmacokinetic/pharmacodynamic model of simulated endocardial vegetations. Antimicrob Agents Chemother 56:5709–5714. https://doi.org/10.1128/AAC.01185-12.
26. Werth BJ, Steed ME, Ireland CE, Tran TT, Arias CA, Rose WE, Sakoulas G, Nonejuie P, Pogliano J, Rybak MJ. 2013. Evaluation of the daptomycin (DAP) mutant selection window in vancomycin resistant enterococci (VRE) in a pharmacokinetic/pharmacodynamic (PK/PD) model of simulated endocardial vegetations (SEV). Interscience Conference on Antimicrob Agents Chemother, Denver, CO.
27. Blaser J. 1985. In-vitro model for simultaneous simulation of the serum kinetics of two drugs with different half-lives. J Antimicrob Chemother 15(Suppl A):125–130. https://doi.org/10.1093/jac/15.suppl_A.125.
28. Saravolatz LD, Stein GE, Johnson LB. 2011. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant Staphylococcus aureus. Clin Infect Dis 52:1156–1163. https://doi.org/10.1093/cid/cir147.
29. Genentech. 2010. Rocephin (ceftriaxone for injection USP) package insert. Genentech USA, Inc., San Francisco, CA.