Pharmacodynamics of Cefepime Alone and in Combination with Various Antimicrobials against Methicillin-Resistant *Staphylococcus aureus* in an In Vitro Pharmacodynamic Infection Model

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Treatment options for gram-positive resistant bacteria are limited; therefore, efforts to evaluate therapy options in the critical care population are warranted. Cefepime has broad-spectrum activity against gram-negative and gram-positive organisms. We have previously demonstrated that the combination of cefepime with vancomycin, linezolid, or quinupristin-dalfopristin had an improved or enhanced effect against methicillin-resistant *Staphylococcus aureus* (MRSA). We investigated various regimens of cefepime alone and in combination against two clinical MRSA isolates (R2481 and R2484) in an established in vitro pharmacodynamic model. Human pharmacokinetic regimen simulations were as follows: cefepime, 2 g every 8 h (q8h) (C8) and 12 h (C12), continuous-infusion 2-g loading dose followed by 4 g alone or in combination with gentamicin and tobramycin (1.0 or 2.0 [G1 and G2 or TB1 and TB2] mg/kg of body weight q12h and 5.0 [G5 or TB5] mg/kg q24h), arbekacin (ARB) (100 mg q12h), linezolid (LIN) (600 mg q12h), tigecycline (TIG) (100 mg q24h), or daptomycin (DAP) (6 mg/kg q24h) for 48 h. The MICs for cefepime, gentamicin, tobramycin, ARB, LIN, TIG, and DAP for the two clinical MRSA isolates (R2481 and R2484) were 4 and 4, 0.25 and 0.5, 128 and 0.5, 0.5 and 0.125, 2 and 4, 0.25 and 0.25, and 0.0625 and 0.125 μg/ml, respectively. At 48 h, combinations of C12 and C8 plus ARB, G1, or G5 (range, -2.05- to -4.32-$\log_{10}$ decrease) demonstrated enhanced lethality against R2481 (resistant to tobramycin) ($P < 0.05$). A similar relationship was demonstrated against R2484 with cefepime plus ARB, gentamicin, or tobramycin (range, -2.05- to -3.63-$\log_{10}$ decrease) ($P < 0.05$). A 99.9% kill was achieved with cefepime plus aminoglycoside combinations as early as 2 h and maintained throughout the 48-h period. TIG was antagonistic when combined with C12 against both isolates. DAP alone achieved 99.9% kill for up to 48 h for both isolates and was the most active agent against R2481 and R2484 (-2.89- and -3.61-$\log_{10}$ decrease at 48 h); therefore, combination therapy did not enhance lethality. Overall, the most potent combinations noted were cefepime in combination with low- and high-dose aminoglycosides. Further investigations with combination therapies are warranted.

Gram-positive organisms, such as *Staphylococcus aureus*, are among the most common bacteria infecting patients in the intensive care units (13). Methicillin-resistant *S. aureus* (MRSA) is a major nosocomial pathogen that has caused severe morbidity and mortality worldwide (38). This organism is endemic in many hospitals and account for as much as 52% of all clinical isolates (43). Until recently, glycopeptide antibiotics, such as vancomycin, were often the only available treatment. The increase in vancomycin usage over the years and the development of resistance to this drug have led to the search for alternative therapy. (16).

Empirical therapy in the intensive care unit (ICU) should be broad enough to cover pathogens such as *Pseudomonas aeruginosa* and *S. aureus*, especially empirically until results of cultures are available (13, 17, 21, 22). In view of the rising prevalence of multidrug-resistant *S. aureus*, combination therapy may be useful in providing a greater antimicrobial empirical coverage and improving efficacy and in helping to prevent the further emergence of resistant organisms (39, 48).

Cefepime is a broad-spectrum cephalosporin active against many gram-negative and gram-positive organisms, including *S. aureus*. Data suggest that time above the MIC is the pharmacodynamically linked parameter that predicts cefepime's efficacy in human and animal models (14). This parameter also has been used as the basis to support consideration of administration of cephalosporin antibiotics by continuous infusion. Cefepime has high affinity for the penicillin-binding proteins of gram-negative and gram-positive bacteria and thus possesses excellent antibacterial activity against methicillin-susceptible *S. aureus*. Maximal efficacy for cephalosporins against *S. aureus* has been demonstrated at 40 to 50% of the dosing interval when serum levels are above MIC with animal infection models (14).

Previous investigations have evaluated the usefulness of various combinations of antibiotics against MRSA, including combinations of quinupristin-dalfopristin and cefepime, linezolid, doxycycline, and vancomycin(3). Several studies have demonstrated additive or synergistic activity when cefepime was combined with various antibiotics, such as vancomycin plus

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β-lactams, ampicillin-sulbactam ± arbekacin, and trimetho-prim-sulfamethoxazole (3, 9, 26, 32, 47). Cefepime is commonly used in combination with an aminoglycosides for empirical therapy in the ICU for gram-negative organisms or for definitive treatment of *P. aeruginosa* infections in critical care settings (22). However, to date, there are limited data regarding the utility of combination therapy with cefepime for empirical treatment of gram-positive infections. We, therefore, evaluated cefepime alone and in combination with gentamicin, arbekacin, tobramycin, linezolid, tigecycline, or daptomycin against MRSA.

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MATERIALS AND METHODS

Bacterial strains. Two clinical strains of MRSA (R2481 and R2484) obtained from William Beaumont Hospital, Royal Oak, Mich., were utilized in all experiments.

Antimicrobial agents. Cefepime (Elan Pharmaceuticals, Americas, San Diego, Calif.), gentamicin (Sigma Chemical Company, St. Louis, Mo.), and linezolid (Pharmacia-Upjohn Laboratories, Kalamazoo, Mich.) were commercially purchased. Arbekacin (Meiji Seika Kaisha, Ltd. Pharmaceutical Division, Tokyo, Japan) (49), daptomycin (Cubist Pharmaceuticals Inc., Lexington, Mass.), and tigecycline (Wyeth Research, Pearl River, N.Y.) were supplied by their respective manufacturers. Stock solutions of each antibiotic were freshly prepared on the day of the experiments and stored in the freezer at −4°C.

Medium. All susceptibility testing and in vitro pharmacodynamic models used Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with 25 mg of calcium/liter and 12.5 mg of magnesium/liter (SMHB). Mueller-Hinton broth adjusted to physiologic ionized calcium concentrations (1.1 to 1.7 mmol/liter) according to NCCLS guidelines (daptomycin dependency on calcium for its mechanism of action) and 12.5 mg of magnesium/liter were used for all daptomycin experiments. Colony counts for all samples were determined by using tryptic soy agar (TSA) (Difco Laboratories) plates.

In vitro susceptibility. MICs and minimum bactericidal concentrations (MBCs) were determined by using a broth microdilution technique with an inoculum of 5×10^6 CFU/ml according to NCCLS guidelines (31).

In vitro pharmacodynamic model. The in vitro pharmacodynamic model consists of a 250-ml one-compartment glass chamber with multiple ports for the removal of SMHB, delivery of antibiotics, and collection of bacterial and antimicrobial samples was utilized (3). All experiments were conducted over 48 h and were performed in duplicate to ensure reproducibility. Prior to each experiment, bacterial colonies from an overnight growth on TSA were added to SMHB to obtain a suspension corresponding to a 0.5 McFarland standard. Then, 2.5 ml of this suspension was added to each of the pharmacodynamic models to produce a starting inoculum of 10^6 CFU/ml. The model was placed in a 37°C water bath for the duration of the experiment, with magnetic stir bars in the medium to allow for continuous mixing. A peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, Ill.) was used to continually replace antibiotic-containing medium with fresh SMHB (at a rate simulating the half-lives of the antibiotics). All antimicrobials were infused over approximately 1 min. Regimen simulations were as follows: cefepime, 2 g every 8 h (q8h) and q12h (estimated peak concentration and half-life of cefepime, 130 μg/ml; 2 h); cefepime continuous infusion (CI), 2-g loading dose followed 4 g q24h (20 μg/ml; 2 h) (6, 10); gentamicin and tobramycin, 1.0 and 2.0 mg/kg q12h (3 and 6 μg/ml; 3 h) and 5 mg/kg q24h (15 μg/ml; 3 h); arbekacin, 100 mg q12h (8 μg/ml; 3 h); linezolid, 600 mg q12h (18 μg/ml; 6 h); tigecycline, 100 mg q24h (1.17 μg/ml; 31 h) (34, 35, 36, 51); daptomycin, 6 mg/kg q24h (6.65 μg/ml simulated free concentration based upon 93% protein binding; 8 h) (2, 24, 50). The elimination rate for all combination models was set for the antibiotic with the shorter half-life, and the antibiotic with a longer half-life was supplemented (8).

Antibiotic assays. Gentamicin, tobramycin, and arbekacin concentrations were determined by using a fluorescence polarization immunoassay (TDx Assay; Abbott Laboratories, Irving, Tex., and Dainabot Co., LTD, Tokyo, Japan). Coefficients of variation for gentamicin, tobramycin, and arbekacin were ≤3.6, ≤4, and ≤5.1%, respectively. The limits of detection for gentamicin, tobramycin, and arbekacin were 1.0, 1.0, and 2.0 μg/ml, respectively. Since the standards for these commercial kits are prepared in serum, we determined the accuracy of detection of aminoglycoside concentrations in SMHB by preparing sets of five spiked samples in both serum and broth for each aminoglycoside in triplicate. The results indicated that there were no appreciable differences demonstrated between serum and broth (1:1.01 to 1:1.06) for arbekacin, gentamicin, and tobramycin, respectively. Linezolid concentrations were determined at the Division of Infectious Diseases at the National Jewish Medical and Research Center (Denver, Colo.), which was previously described by Allen et al. (3). The standard curve for linezolid in SMHB ranged from 0.5 to 30 μg/ml. The within-sample precision (percent coefficient of variation) of validation for a single standard concentration was 0.69%, and the overall validation precision across all standards was 1.04 to 4.39. The limit of detection for linezolid was 0.5 μg/ml (3).

Concentrations of all other agents were determined by using standard agar diffusion bioassay procedures. Cefepime and daptomycin concentrations were determined by using antibiotic assay medium 5 with *Micrococcus luteus* ATCC 9341 and antibiotic assay medium 1 supplemented with 50 mg of calcium/liter and *M. luteus* ATCC 9341, respectively. Tigecycline concentrations were determined by using agarose medium and *Bacillus cereus* ATCC 11778 spore suspension. Standards and samples were tested in triplicate using blank 1/4-in. disks saturated with 20 μl of the appropriate solution. Concentrations of 150, 130, 20, 8, and 2 μg/ml, 10, 7.5, 5, 2.5, and 1.25 μg/ml, and 4, 2, 1, 0.5, 0.25, 0.12, and 0.06 μg/ml were used as standard curves for cefepime, daptomycin, and tigecycline, respectively. Coefficients of variation for cefepime, daptomycin, and tigecycline assays were <10, ≤8, and 8.4%, respectively. The limits of detection for each of the above assays were 2.0 μg/ml (cefepime), 1.25 μg/ml (daptomycin), and 0.06 μg/ml (tigecycline).

Pharmacokinetic analysis. Antibiotic concentrations were determined from samples drawn in duplicate from each model at 0, 0.5, 1, 2, 4, 6, 8, 24, 28, 32, and 48 h. Samples were stored at −70°C until analysis. Antibiotic peak, trough concentrations, and half-life were calculated using concentration-time plots of the model samples. The area under the concentration-time curve from 0 to 24 h was calculated by using the linear trapezoid method and the PKANALYST program (version 1.10; MicroMath Scientific Software, Salt Lake City, Utah).

Pharmacodynamic analysis. Samples (approximately 1.5 ml each) from each model were collected at 0, 1, 2, 4, 6, 8, 24, 28, 32, and 48 h. Samples were then serially diluted in 0.9% sodium chloride. Bacterial counts were determined by plating 100-μl samples of each diluted sample on TSA, using an automated spiral dispenser (Automatic Spiral Plater; Don Whitley Scientific Limited, West Yorkshire, England). All samples were diluted 10- to 100-fold before plating in order to minimize antibiotic carryover or by plating or vacuum filtration (sample washed through a 0.22-μm-pore-size filter with normal saline) when sample with predicted concentrations were close to the MIC for the experiment organisms. Plated samples were then incubated at 37°C for 24 h, and colony counts (log_{10} CFU/ml) were determined by using a laser bacteria colony counter (ProtoCOL, version 2.05.02; Synbiosis, Cambridge, England). The limit of detection for this method of colony count determination was 2-log_{10} CFU/ml. In vitro model time-kill curves were determined by plotting mean colony counts (log_{10} CFU/ml) from each model versus time. Bactericidal activity (99.9% kill) was defined as a ≥3-log_{10} CFU/ml reduction in colony count from the starting inoculum. Enhancement of activity was defined as an increase in kill of ≥2-log_{10} CFU/ml by combination of antimicrobials versus the most active single agent of that combination (3). Improvement was defined as >1- and <2-log_{10}-CFU/ml increase in kill in comparison to the most active single agent, while combinations that resulted in ≥1-log_{10} bacterial growth in comparison to that with the least-active single agent were considered to represent antagonism (3). The terms "improvement" and "enhancement" were used because our simulations used therapeutically obtained serum concentrations, and this did not permit the mathematical modeling necessary to consider the standard terms "additivity" and "synergy" (3). "Indifferent" was defined as no differences. Reductions in colony counts and the ability to achieve a 99.9% kill were determined over a 48-h period and were compared between regimens.

Detection of resistance. Samples (100 μl each) from each time point were plated onto TSA containing an antibiotic concentration of four and eight times the MIC for each organism and were incubated for 48 h at 37°C in order to monitor the development of resistance. Plates were visually inspected for growth of resistant subpopulations after 24, 32, and 48 h of incubation. The MIC for each organism was determined by using broth microdilution methods.

Statistical analysis. Differences between regimens (log_{10} CFU/ml) at 24 and 48 h were determined using one-way analysis of variance with Tukey's post-hoc test. For all experiments, a P value of ≤0.05 was considered indicative of statistical significance. All statistical analyses were performed using SPSS (version 11.5; SPSS, Inc., Chicago, Ill.) (3).

TABLE 1. Susceptibilities of the two MRSA clinical strains

Antimicrobial agent	Activity (μg/ml) against strain:			
	MRSA R2481		MRSA R2484	
	MIC	MBC	MIC	MBC
Cefepime	4	8	4	8
Gentamicin	0.25	0.5	0.5	1
Tobramycin	128	256	0.5	1
Arbekacin	0.5	0.5	0.13	1
Daptomycin	0.06	0.13	0.13	0.5
Linezolid	2	32	4	16
Tigecycline	0.25	0.5	0.25	0.5

RESULTS

Susceptibility testing. The microdilution MICs and MBCs for all antimicrobials tested are shown in Table 1. Both strains, MRSA R2481 and R2484, were susceptible to all the antimicrobials tested, except tobramycin. MRSA R2484 was tobramycin susceptible, and MRSA R2481 was tobramycin resistant (MIC = 128 μg/ml).

Pharmacokinetics. Observed pharmacokinetic parameters achieved in the in vitro model experiments are listed in Table 2.

Pharmacodynamics. The reduction in bacterial inoculum for each antimicrobial alone is shown in Table 3, and combination results are shown in Tables 4 and 5. Although there appeared to be some improvement in kill as a function of dosing interval (greatest effect with CI, followed by q8h dosing, followed by q12h dosing) for cefepime, this was not statistically significant. Daptomycin alone demonstrated the greatest activity against both MRSA isolates, and therefore, no enhanced or improved efficacy in combination was observed. Gentamicin and tobramycin alone did not demonstrate kill against both MRSA isolates at 48 h; however, a 99.9% kill was achieved at earlier time points. Linezolid and tigecycline were each bacteriostatic when administered alone, and no time point examined has achieved a 99.9% kill. We observed no effective activity for tobramycin alone and in combination with cefepime against R2481 due to the resistant susceptibility profile (data not shown).

The results of combination regimens of inoculum changes for 48-h pharmacodynamic models for the tested strains are

TABLE 3. Inoculum changes over 48 h obtained for each antimicrobial alone

Antimicrobial agent	Change over 48 h (log$_{10}$ CFU/ml) for strain:	
	MRSA R2481	MRSA R2484
Cefepime (2g q12h)	+1.51	+1.39
Cefepime (2g q8h)	−0.63	−1.83
Cefepime (continuous infusion)	−1.08	−2.40
Gentamicin (5.0 mg/kg q24h)	−0.73	−1.70
Gentamicin (2.0 mg/kg q12h)	−2.09	−2.84
Gentamicin (1.0 mg/kg q12h)	+0.28	+0.44
Tobramycin (5.0 mg/kg q24h)	—[a]	−1.06
Tobramycin (2.0 mg/kg q12h)	+2.18	−0.75
Tobramycin (1.0 mg/kg q12h)	—	−0.35
Arbekacin	−1.56	−1.06
Daptomycin	−2.89	−3.61
Linezolid	−2.65	−1.78
Tigecycline	−0.37	−0.72

[a] —, model was not performed.

depicted in Fig. 1. Positive values indicate regrowth. Against R2481, significant enhancement of efficacy was observed with cefepime (CI), cefepime (q8h and q12h) with gentamicin (1 mg/kg q12h and 5 mg/kg q24h) or arbekacin ($P \leq 0.04$). Significant enhancement of efficacy was also demonstrated against R2484 with cefepime (q8h and q12h) and both gentamicin (1 mg/kg q12h and 5 mg/kg q24h) and tobramycin (1.0 and 2 mg/kg q12h and 5.0 mg/kg q24h) or arbekacin (q12h only) ($P \leq 0.02$). A 99.9% kill was achieved at 8, 24, and 48 h for the following combinations against both strains: cefepime, q12h, q8h, and CI, with gentamicin and tobramycin (5.0 mg/kg q24h), gentamicin and tobramycin (2.0 mg/kg q12h), or arbekacin. Cefepime (CI) was very active against R2484; therefore, no enhancement of effect was demonstrated against R2484 versus that of cefepime (CI) with any of the tested antimicrobials. Improved activity was demonstrated against R2481 with cefepime (q8h) plus gentamicin (2.0 mg/kg q12h) or cefepime (CI) plus gentamicin (1.0 and 2.0 mg/kg q12h), gentamicin (5.0 mg/kg q24h), or tigecycline ($P > 0.05$). Against R2484, improved activity was demonstrated with cefepime (q12h) plus linezolid or cefepime (q8h) plus arbekacin as well as cefepime (CI) plus gentamicin and tobramycin (1.0 mg/kg q12h), tobra-

TABLE 2. Pharmacokinetic parameters

Antimicrobial agent (regimen)	Peak (μg/ml)	Trough (μg/ml)	Half-life (h)
Cefepime (q12H)	140 ± 21.5	2.5 ± 0.69	2.0 ± 0.22
Cefepime (q8H)	137.4 ± 2.97	9.0 ± 0.19	2.0 ± 0.04
Cefepime (LD[a] + continuous infusion)	136.8 ± 6.60	19.4 ± 1.40	N/A[b]
Gentamicin (5.0 mg/kg q24H)	14.7 ± 0.50	0.21 ± 0.01	2.6 ± 0.04
Gentamicin (2.0 mg/kg q12H)	5.4 ± 0.09	0.46 ± 0.06	3.2 ± 0.07
Gentamicin (1.0 mg/kg q12H)	2.5 ± 0.09	0.11 ± 0.01	3.0 ± 0.06
Tobramycin (5.0 mg/kg q24H)	15.4 ± 0.08	0.41 ± 0.01	3.1 ± 0.04
Tobramycin (2.0 mg/kg q12H)	6.2 ± 0.05	0.29 ± 0.01	2.6 ± 0.04
Tobramycin (1.0 mg/kg q12H)	3.15 ± 0.03	0.26 ± 0.01	3.2 ± 0.10
Arbekacin	7.9 ± 0.40	0.63 ± 0.08	3.3 ± 0.17
Daptomycin	6.8 ± 0.12	0.69 ± 0.10	7.3 ± 0.35
Linezolid	21.6 ± 1.00	4.0 ± 0.01	5.7 ± 0.35
Tigecycline	1.2 ± 0.02	0.69 ± 0.01	32 ± 2.0

[a] LD, loading dose.
[b] N/A, not applicable.

TABLE 4. R2481 versus cefepime combined with various antimicrobials at various dosing intervals

Antimicrobial agent (regimen)	Activities of drug against MRSA R2481 in combination with[a]:					
	Cefepime (2 g q12h)		Cefepime (2 g q8h)		Cefepime (continuous infusion)	
	Δ48-h $\log_{10}$ CFU/ml	Activities	Δ48-h $\log_{10}$ CFU/ml	Activities	Δ48-h $\log_{10}$ CFU/ml	Activities
Gentamicin (5.0 mg/kg q24h)	−2.68**	ENC	−2.58*	ENC	−1.69*	IMP
Gentamicin (2.0 mg/kg q12h)	−1.69	IMP	−1.34	IMP	−1.54	IMP
Gentamicin (1.0 mg/kg q12h)	−3.82**	ENC	−3.14**	ENC	−2.62***	ENC
Arbekacin	−2.23*	ENC	−2.34*	ENC	−2.34***	ENC
Daptomycin	0.04	IND	−0.85	IND	−0.11	IND
Linezolid	−0.39	IND	−0.56	IND	−0.15	IND
Tigecycline	1.75	ANG	−0.03	IND	−1.86	IMP

[a] Change in 48-h $\log_{10}$ CFU of R2481/ml with cefepime (2g q12h, 2 g q8h, and continuous infusion) plus gentamicin (1.0 or 2.0 mg/kg q12h or 5.0 mg/kg q24h), arbekacin, linezolid, daptomycin, or tigecycline compared to results with the most potent agent alone. ENC, enhancement; IMP, improvement; ANG, antagonistic; IND, indifferent. *, $P \le 0.05$; **, $P \le 0.01$; ***, $P \le 0.001$.

mycin (2.0 mg/kg), or arbekacin ($P \le 0.009$). A 99.9% kill was achieved at 8, 24, and 48 h for the following combinations against both strains: cefepime (q8h) plus gentamicin (2.0 mg/kg q12h) or plus arbekacin and cefepime (CI) plus gentamicin (5.0 mg/kg q24h), plus gentamicin and tobramycin (2.0 mg/kg q12h), plus gentamicin and tobramycin (1.0 mg/kg q12h), or plus tigecycline. Antagonism was noted only with cefepime (2 g q12h) plus tigecycline.

Detection of resistance. Resistance was detected for tobramycin (12-fold the MIC) for monotherapy regimens. No evidence of resistance was observed with any other regimen tested, including models where significant bacterial regrowth occurred.

DISCUSSION

The inappropriate use of antibiotics has contributed to the emergence of resistance globally with gram-negative bacilli and gram-positive bacteria (28). Infections with antibiotic-resistant organisms are associated with increased mortality, increased length of stay in the ICU and hospital, and increased costs compared to infections with antibiotic-susceptible organisms (28). Some of the most problematic organisms are *P. aeruginosa* and MRSA (17, 20, 21, 22). Appropriate empirical choices in ICU settings are critical, due to the rise in multi-

drug-resistant *P. aeruginosa* and MRSA (28). The prevalence of MRSA has been reported to be as high as 52% in this setting (43). Empirical ICU coverage often comprises combinations of broad-spectrum antipseudomonal β-lactams, aminoglycosides (such as gentamicin, tobramycin, and amikacin), arbekacin (available in Japan only), and/or a glycopeptide (vancomycin and teicoplanin) or monotherapy with a potent broad-spectrum agent (such as the carbapenems) (22, 49, 52). Combination therapy is often recommended for empirical treatment of ICU infections, since monotherapy, even with a highly active agent, may not cover all potential organisms, and the emergence of resistant organisms is always a potential (22). Specific empirical combination therapy for *S. aureus* has not been advocated.

Cefepime is a broad-spectrum cephalosporin that offers broad activity against both gram-negative organisms, including *P. aeruginosa*, and gram-positive organisms, such as *S. aureus* (19, 42). In vitro data have suggested that the bactericidal activity of β-lactams is generally concentration independent, with a significant postantibiotic effect against gram-positive bacteria (14). Attempts have been made to establish optimal dosing regimens, and intermittent dosing continues to be the standard of practice. Though shorter intermittent dosing intervals will optimize the pharmacodynamic properties of most β-lactams, this is highly dependent on the organism and the

TABLE 5. R2484 versus cefepime combined with various antimicrobials at various dosing intervals

Antimicrobial agent (regimen)	Activities of drug against MRSA R 2484 in combination with[a]:					
	Cefepime (2 g q12h)		Cefepime (2 g q8h)		Cefepime (continuous infusion)	
	Δ48-h $\log_{10}$ CFU/ml	Activities	Δ48-h $\log_{10}$ CFU/ml	Activities	Δ48-h $\log_{10}$ CFU/ml	Activities
Gentamicin (5.0 mg/kg q24h)	−3.85*	ENC	−2.05	ENC	−1.46**	IMP
Gentamicin (2.0 mg/kg q12h)	0.23	IND	−0.93	IND	−0.93	IND
Gentamicin (1.0 mg/kg q12h)	−4.32***	ENC	−2.05	ENC	−1.46**	IMP
Tobramycin (5.0 mg/kg q24h)	−2.81**	ENC	−2.05	ENC	−0.44	IND
Tobramycin (2.0 mg/kg q12h)	−3.03***	ENC	−2.05	ENC	−1.46**	IMP
Tobramycin (1.0 mg/kg q12h)	−3.63***	ENC	−2.05	ENC	−1.46**	IMP
Arbekacin	−2.9**	ENC	−1.24	IMP	−1.46**	IMP
Daptomycin	0.9	IND	0.69	IND	0	IND
Linezolid	−1.32	IMP	−0.43	IND	−0.24	IND
Tigecycline	2.06	ANG	−0.28	IND	−0.66	IND

[a] Change in 48-h $\log_{10}$ CFU of R2484/ml with cefepime (2 g q12h, 2 g q8h, and continuous infusion) plus gentamicin (1.0 or 2.0 mg/kg q12h or 5.0 mg/kg q24h), tobramycin (1.0 or 2.0 mg/kg q12h or 5.0 mg/kg q24h), arbekacin, daptomycin, linezolid, or tigecycline compared to results with the most potent agent alone. ENC, enhancement; IMP, improvement; ANG, antagonistic; IND, indifferent. *, $P \le 0.05$; **, $P \le 0.01$; ***, $P \le 0.001$.
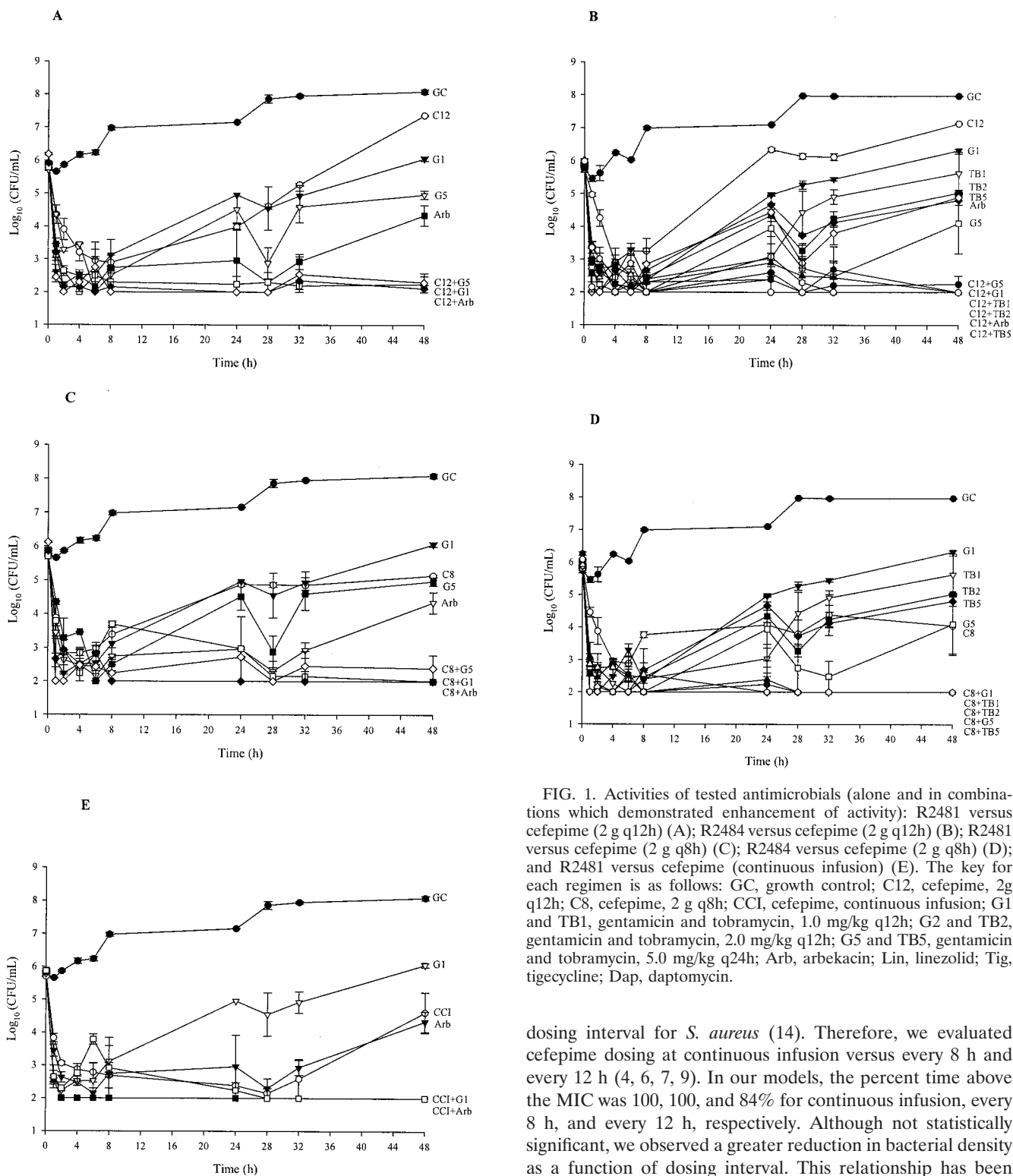
FIG. 1. Activities of tested antimicrobials (alone and in combinations which demonstrated enhancement of activity): R2481 versus cefepime (2 g q12h) (A); R2484 versus cefepime (2 g q12h) (B); R2481 versus cefepime (2 g q8h) (C); R2484 versus cefepime (2 g q8h) (D); and R2481 versus cefepime (continuous infusion) (E). The key for each regimen is as follows: GC, growth control; C12, cefepime, 2g q12h; C8, cefepime, 2 g q8h; CCI, cefepime, continuous infusion; G1 and TB1, gentamicin and tobramycin, 1.0 mg/kg q12h; G2 and TB2, gentamicin and tobramycin, 2.0 mg/kg q12h; G5 and TB5, gentamicin and tobramycin, 5.0 mg/kg q24h; Arb, arbekacin; Lin, linezolid; Tig, tigecycline; Dap, daptomycin.

specific antimicrobial agent (4, 10, 43, 44, 45). Time above the MIC is the most important pharmacodynamic parameter for predicting the outcome (27). The maximal efficacy for cephalosporins in animal infection models is observed when serum concentrations are greater than the MIC for 40 to 50% of

dosing interval for *S. aureus* (14). Therefore, we evaluated cefepime dosing at continuous infusion versus every 8 h and every 12 h (4, 6, 7, 9). In our models, the percent time above the MIC was 100, 100, and 84% for continuous infusion, every 8 h, and every 12 h, respectively. Although not statistically significant, we observed a greater reduction in bacterial density as a function of dosing interval. This relationship has been previously shown with cefepime against gram-negative organisms (6, 9, 11, 12, 41).

The observed activity of cefepime against MRSA in this study may be related to some minimal activity against penicillin-binding protein 2a and/or due to a heteroresistance profile for beta-lactams in which some initial activity may be expected against a portion of the bacterial inoculum (40). We retrospec-

tively performed subpopulation analysis versus oxacillin for both strains utilized in this experiment and compared the results to a strain (COL) that demonstrates a homogenous susceptibility profile for oxacillin (data not shown). As expected, both R2481 and R2484 demonstrated a heterogeneous profile for oxacillin. Although the activity of cefepime against MRSA is minimal, it has been observed that improved activity against MRSA can be demonstrated when cefepime is combined with vancomycin, quinupristin-dalfopristin, and linezolid (3, 17). Our data demonstrated improved kill with cefepime in combination with linezolid against R2484 which is similar to that reported by Allen et al. In this study, enhanced kill with cefepime plus vancomycin and improved kill with quinupristin-dalfopristin was also demonstrated. Raymond et al. obtained similar results with cefpirome ("fourth-generation" broad-spectrum cephalosporin structurally similar to cefepime) plus vancomycin against MRSA (37).

In our study, the most potent activity against MRSA was observed when cefepime was combined with an aminoglycoside. β-Lactams plus aminoglycoside have traditionally demonstrated synergy and additivity for a variety of pathogens, including *P. aeruginosa*, *Enterobacteriaceae*, and *Acinetobacter* (12, 29, 30, 33, 43, 46). We found that combinations of cefepime plus an aminoglycoside administered every 12 h or every 24 h demonstrated the most potent activity.

Cefepime is a broad-spectrum antibiotic commonly employed in critical care settings. In a recent study by Konstantinou et al. examining the impact of cefepime in an ICU setting, less resistance to therapy, less vancomycin-resistant enterococci, reduced vancomycin use, and shorter posttherapy hospitalization were observed with patients treated with cefepime than with those treated with ceftazidime (23). Araake et al. examined cefepime in combination with arbekacin or vancomycin and teicoplanin against a mixed culture of MRSA and *P. aeruginosa* (5). They demonstrated enhancement of effect with cefepime plus arbekacin against MRSA and *P. aeruginosa* but did not demonstrate enhancement with cefepime plus vancomycin or teicoplanin against MRSA (5). They suggested that arbekacin alone demonstrated good bactericidal activity and that combination with cefepime enhanced the bactericidal activity against MRSA (5).

A potential limitation of our research is the use of therapeutic concentrations only of all tested agents (1). This is in contrast to traditional synergy testing, such as checkerboard and killing-curve techniques, which are commonly conducted using a range of subinhibitory concentrations of one or both antimicrobials. While this typically leads to identification of a wider range of synergistic combinations, we chose to study clinically achievable concentrations in order to allow broader applicability of our results to clinical practice (1).

Despite this potential limitation, our data suggest that cefepime in combination with aminoglycoside offers reasonable coverage of MRSA if the isolate is susceptible to the aminoglycosides. Preliminary in vitro models (data not shown) performed with tobramycin and cefepime in combination against the tobramycin-resistant isolate (R2481) did not demonstrate significant activity against this isolate; therefore, additional combination studies against this pathogen were not pursued. Although we did not confirm the presence of an aminoglycoside-modifying enzyme in this organism, the ami-

noglycoside-modifying enzymes in MRSA that are most responsible for tobramycin resistance are the aminoglycoside-6-*O*-nucleotidyltransferase I and aminoglycoside-6′-*N*-acetyltransferase/2″-*O*-phosphoryltransferase (18, 25). The prevalence of MRSA resistant to aminoglycosides has been noted to be as high as 35.5% in a recent surveillance study, and this factor should be noted when applying this data to clinical setting (15).

In the present study we were able to show improved or enhanced activity through the use of a variety of antimicrobial combinations encompassing cefepime, gentamicin, tobramycin, arbekacin, and linezolid against MRSA. The combination of cefepime and an aminoglycoside against MRSA is encouraging; however, caution should be applied until these results can be verified clinically.

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