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Pharmacodynamics of RWJ-54428 against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* in a Neutropenic Mouse Thigh Infection Model[▽]

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RWJ-54428 (also known as MC-02,479) is a new cephalosporin with promising activity against gram-positive bacteria. The pharmacodynamics (PDs) of RWJ-54428 against *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* were studied in a neutropenic mouse thigh infection model. The RWJ-54428 MICs ranged from 0.25 to 1 mg/liter. Mice with ca. 10^6 CFU/thigh at the initiation of therapy were treated intraperitoneally with RWJ-54428 at doses that ranged from 3 to 1,200 mg/kg of body weight/day (in 2, 3, 4, 6, or 12 divided doses) for 24 h. The maximal reductions in bacterial counts in thigh tissues at 24 h for the methicillin-resistant *S. aureus*, penicillin-resistant *S. pneumoniae*, and *E. faecalis* strains were −2.8, −3.8, and −1.7 $\log_{10}$ CFU/thigh, respectively. The percentage of a 24-h dosing interval that the unbound serum RWJ-54428 concentrations exceeded the MIC ($fT_{>MIC}$) was the pharmacokinetic (PK)-PD parameter that best described the efficacy of RWJ-54428. The $fT_{>MIC}$s for a bacteriostatic effect (no net change in the numbers of CFU/thigh over 24 h) ranged from 14 to 20% for staphylococci and streptococci; for maximal reductions in the numbers of CFU/thigh, the $fT_{>MIC}$s ranged from 22 to 36% for these strains. For *E. faecalis*, the ranges of $fT_{>MIC}$s for static and maximal effects were 30 to 46% and 55 to 60%, respectively. These data show that treatment with RWJ-54428 results in marked antibacterial effects in vivo, with the PK-PD parameters for efficacy being comparable to those for the efficacy of penicillins and carbapenems active against staphylococci and pneumococci.

RWJ-54428 is a novel cephalosporin with good potency in vitro and in vivo against *Staphylococcus aureus* (including methicillin-resistant *S. aureus* [MRSA] and glycopeptide-intermediate isolates), *Streptococcus pneumoniae* (including penicillin-resistant *S. pneumoniae* isolates), and *Enterococcus faecalis* (3). Pharmacokinetic (PK)-pharmacodynamic (PD) studies with animal models of infection are useful for targeting the exposures associated with optimal activity. The PK-PD relationships determined in animal models of infection can be used to establish the dosage regimens for testing with humans (4, 5). Previous studies with β-lactams have established that the percentage of a 24-h dosing interval that the free drug concentrations exceed the MIC ($fT_{>MIC}$s) is the PK-PD parameter best linked with efficacy (10, 13). For these studies, we wanted to confirm that the PK-PD parameter that best describes the efficacy of RWJ-54428 was indeed $fT_{>MIC}$ and determine the magnitude of that parameter required to achieve the maximal bactericidal effects against gram-positive pathogens.

(The work described in this paper was conducted as part of a research collaboration with R. W. Johnson Pharmaceutical Research and Development. This work was presented in part at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, September 1999.)

MATERIALS AND METHODS

Antimicrobial agents. RWJ-54428 was synthesized at Microcide Pharmaceuticals, Inc., Mountain View, CA.

Bacterial strains. MRSA COL and MRSA 076 were provided by Chip Chambers. *S. pneumoniae* ATCC 49619 and *E. faecalis* ATCC 29212 are ATCC reference strains. The following clinical isolates were also used: MRSA 602, 604, and 609; *S. pneumoniae* SP 019; and *E. faecalis* EFS 007.

Susceptibility testing. MICs were determined by a broth microdilution assay, according to CLSI (formerly NCCLS) reference methods (11). MICs were de-

TABLE 1. Relationship between RWJ-54428 MIC and $fT_{>MIC}$ required to achieve various degrees of antibacterial effects in vivo

Organism	MIC (μg/ml)	% $fT_{>MIC}$ of RWJ-54428 required to achieve:			
		Static effect	1-log drop	2-log drop	E_{max}
MRSA COL	1.0	14	20	NA[a]	36
MRSA 076	1.0	16	23	28	32
MRSA 602	0.5	17	20	NA	24
MRSA 604	0.5	20	23	28	30
MRSA 609	0.5	16	18	21	22
S. pneumoniae ATCC 49619	0.03	18	21	23	28
S. pneumoniae SP 019[b]	0.5	16	22	28	30
E. faecalis ATCC 29212	0.06	30	48	NA	60
E. faecalis EFS 007	0.125	46	56	NA	60

[a] NA, not applicable.
[b] Penicillin-resistant strain.

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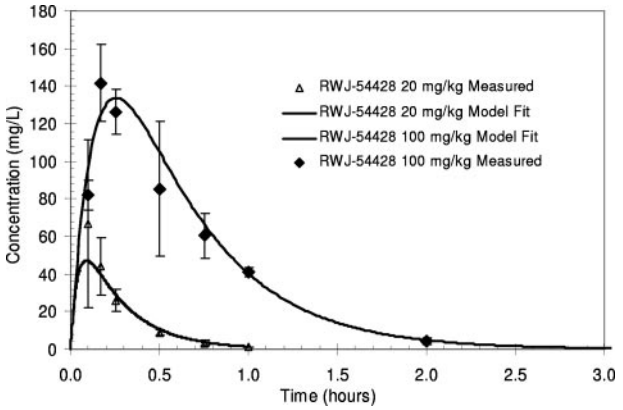

FIG. 1. Serum RWJ-54428 concentrations in mice after a single intraperitoneal dose.

termined in triplicate for the isolates used for inoculation and for the isolates recovered from mouse thighs.

Assays were performed by using a final volume of 100 μl. The inocula were adjusted to yield a final cell density of 5×10^5 CFU/ml. The antibiotics were prepared at a concentration equivalent to twofold the highest desired final concentration in culture medium and were then diluted directly into 96-well microtiter plates by serial twofold dilution. The microtiter plates were incubated for 24 h at 35°C and were read with a plate reader (Molecular Devices, Sunnyvale, CA) at 650 nm as well as by visual observation with a reading mirror.

All procedures were approved by the Microcide Pharmaceuticals Institutional Animal Care and Use Committee, as outlined by the Animal Welfare Act.

Neutropenic mouse thigh model. Male Swiss mice were made neutropenic by the intraperitoneal injection of 150 mg/kg of body weight cyclophosphamide (Cytoxan, Mead Johnson, Princeton, NJ) on days 1 and 4. On day 5, the mice were infected by the intramuscular injection of 0.1 ml of inoculum in each thigh (four thighs per group per time point). The bacteria were grown overnight at 35°C in brain heart infusion broth (BHIB) or Todd-Hewitt broth (THB). On the following morning they were subcultured into fresh BHIB or THB and incubated for 4 h at 35°C. The inocula were adjusted to a final concentration of ~5.0×10^6 CFU/ml. RWJ-54428 was administered intraperitoneally at various time points beginning 2 h postinfection. RWJ-54428 was administered at total daily doses of 3 to 1,200 mg/kg/day divided into 2, 3, 6, or 12 doses. The individual doses ranged from 1.5 to 200 mg/kg. All mice were euthanized after 24 h of therapy by using CO_2, and both thighs were removed aseptically and homogenized in 4 ml of ice-cold phosphate-buffered saline. Serial 10-fold dilutions of the homogenized material were plated on Mueller-Hinton or blood agar, and the colonies were counted. The reduction in bacterial counts was determined by inspection of the log number of CFU/thigh at the start of therapy and comparison to the numbers recovered after 24 h of therapy.

PKs. Male Swiss mice were administered a single intraperitoneal dose of RWJ-54428 at 20 and 100 mg/kg on the fifth day after the initiation of cyclophosphamide treatment (as described above for the thigh infection model). Groups of three mice each were killed at 0.08, 0.16, 0.25, 0.5, 0.75, 1.0, and 2.0 h after dosing. Blood samples (one sample from each animal) were collected by cardiac puncture. Serum concentrations were fit by using the WinNonlin program (Pharsight, Mountain View, CA).

Protein binding. The level of protein binding of RWJ-54428 when it was administered at doses of 25, 50, and 100 mg/liter was determined by ultrafiltration. The serum was prewarmed to 37°C and adjusted to pH 7.4 ± 0.2 with CO_2. Aliquots were loaded into the upper reservoir of Centrifree disposable micropartition units (YM-30; Millipore, Bedford, MA) and centrifuged at 1,900 × *g* for 10 min at 25°C. A solution of 0.2 N acetic acid was mixed with aliquots of the ultrafiltrate prior to high-performance liquid chromatography (HPLC) analysis or, in the case of mouse serum, was added to the bottom reservoir of the ultrafiltration devices prior to centrifugation to prevent drug degradation in the alkaline ultrafiltrate. The protein binding was controlled by the use of a standard curve (1 to 100 μg/ml) prepared with a control sample (25 μg/ml) generated with spiked serum ultrafiltrates and a standard curve prepared with a sample generated by refiltering the spiked ultrafiltrates. The double filtration ensured the same filter membrane recovery of drug as that for the actual serum-binding sample. The percent recovery of RWJ-54428 from the ultrafiltration units was determined by comparison of the peak areas of these nonultrafiltered standards with those of the ultrafiltered standards used to prepare the standard curves (A_N and A_U, respectively), as follows: $(A_U/A_N) \times 100$. The percent serum protein binding was determined as [1 − (RWJ-54428 concentration in serum after ultrafiltration/spiked RWJ-54428 concentration in serum)] × 100.

Bioanalytical assay. The serum and serum ultrafiltrate samples were analyzed for RWJ-54428 by reverse-phase HPLC with UV detection at 254 and 280 nm. The HPLC system used a 5-μm Phenomenex Luna C_{18} column (2.5 mm by 250 mm). The mobile phase consisted of 0.1 M ammonium acetate buffer, pH 6, and acetonitrile; the flow rate was 1.0 ml/min of 90% 0.1 M ammonium acetate, pH 6, and 10% acetonitrile for 5 min, and then 79% 0.1 M ammonium acetate, pH 6, and 21% acetonitrile for 15 min. Serum and serum ultrafiltrate samples were prepared for analysis by using an acid-protein precipitation method. The extraction efficiencies ranged from 80 to 100%. The range of detection of the assay was from 0.5 to 100 mg/liter, and the intraday and interday precisions (coefficients of variation) were in the ranges of 1 to 30 and 1 to 46%, respectively.

PD modeling. The relationship between each of the PK and PD parameters (i.e., $fT_{>MIC}$, the free area under the concentration-time curve [*f*AUC/MIC], and the free maximum concentration in serum/MIC [fC_{max}/MIC]) and the reduction in the log number of CFU/thigh between time zero and 24 h after the start of treatment were analyzed by using the sigmoid maximum-effect (E_{max}) PD model, as follows: change in log CFU/thigh = $[E_{max} \cdot Xg/(EC_{50}g + Xg)] + E_0$, where E_{max} is the maximum reduction in the log number of CFU/thigh, X is the PK-PD parameter being examined (e.g., 24-h AUC/MIC), EC_{50} is the X value corresponding to 50% of the E_{max}, E_0 is the effect when X is equal to 0 (i.e., for the untreated control animals), and g is a sigmoidicity factor which controls the steepness of the curve.

The best model for each data set was established by using the Akaike information criterion (1).

RESULTS

Susceptibility studies. The MICs of RWJ-54428 are shown in Table 1. The MICs for the posttreatment isolates recovered from the mouse thighs did not change after exposure to RWJ-54428 therapy.

Mouse PKs. The total drug serum concentrations of RWJ-54428 following a single intraperitoneal dose in neutropenic mice are shown in Fig. 1. RWJ-54428 was absorbed rapidly after intraperitoneal administration, with the times to C_{max} achieved at 0.09 and 0.26 h for 20 and 100 mg/kg, respectively. The decline in the concentrations was best described by a one-compartment model with first-order absorption. The values for the PK parameters are shown in Table 2. The range of RWJ-54428 serum clearance values was 0.9 to 1.2 liters/h/kg.

PD modeling. At the start of RWJ-54428 treatment, the bacterial titers in the thighs were 5.8 to 6.8, 5.9 to 6.1, and

TABLE 2. Serum PK parameters of RWJ-54428 following a single intraperitoneal injection in male neutropenic mice[a]

RWJ-54428 dose (mg/kg)	Mean wt (kg)	*V*/*F* (liter/kg)	C_{max} (mg/liter)	T_{max} (h)	AUC (mg · h/liter)	CL/*F* (liter/hr/kg)	$t_{1/2}$ (h)	PB (%)
20	0.022	0.3	47.3	0.09	16.6	1.2	0.17	60
100	0.022	0.44	133.3	0.26	108	0.9	0.33	60

[a] *V*/*F*, volume of distribution; T_{max}, time to C_{max}; CL/*F*, clearance; $t_{1/2}$, half-life; PB, protein binding. The other abbreviations are defined in the text.

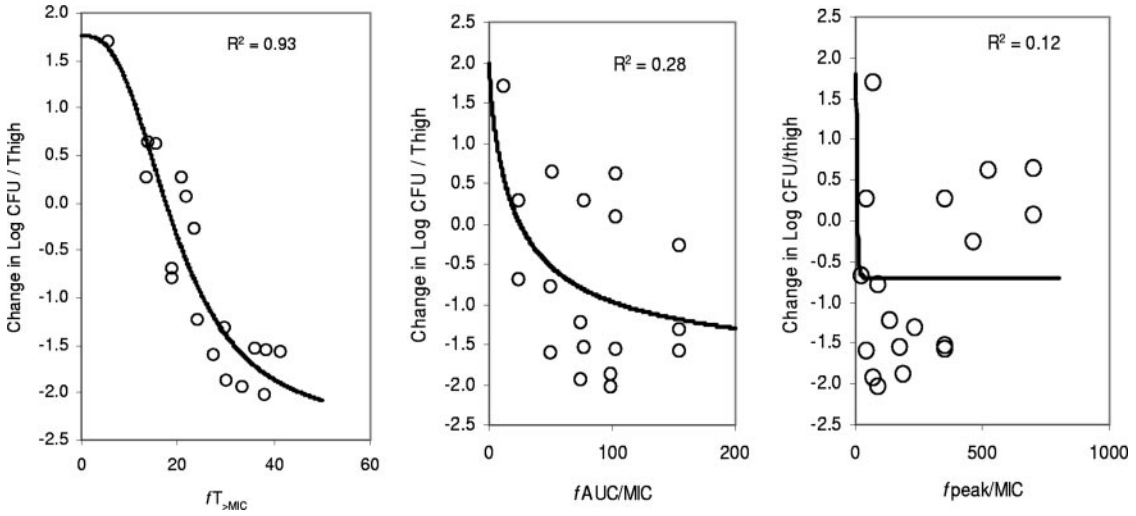

FIG. 2. Relationships between $fT_{>MIC}$, *f*AUC/MIC, and fC_{max}/MIC (*f*peak/MIC) and the change in the $\log_{10}$ number of CFU/thigh of MRSA COL. Each symbol represents the data from four thighs. The lines are the best model fits of the data. R^2 is the correlation coefficient.

5.6 to 6.6 log CFU/thigh for MRSA, *E. faecalis*, and *S. pneumoniae*, respectively. The relationships between the antibacterial effects and each of the PD parameters are shown in Fig. 2 for MRSA COL, Fig. 3 for *S. pneumoniae* SP 019, and Fig. 4 for *E. faecalis* EFS 007. The best relationships were seen when the results were correlated with $fT_{>MIC}$, with R^2 values of 0.93, 0.94, and 0.70 for MRSA COL, *S. pneumoniae* SP019, and *E. faecalis* EFS 007, respectively. The relationships between the RWJ-54428 MICs and the $fT_{>MIC}$s for all strains are shown in Table 1. The maximal bactericidal effects varied between strains and organisms. For MRSA, the maximal bactericidal effects ranged from −1.95 to −2.83 log CFU/thigh, and for *S. pneumoniae*, the maximal bactericidal effects were −2.79 to −3.75 log CFU/thigh; but for *E. faecalis*, the maximal effects were only −1.42 to −1.65 log CFU/thigh.

DISCUSSION

The increasing resistance of gram-positive organisms to all classes of antibacterial agents has limited the therapeutic options for many infections. The high incidence of methicillin resistance in hospitals (as well as its appearance in the outpatient setting), coupled with the emergence of glycopeptide-intermediate *Staphylococcus aureus* strains, has further complicated the prevention and treatment of serious infections due to staphylococci (9, 12). Furthermore, resistance to penicillin among strains of *Streptococcus pneumoniae* has spread worldwide (8), with a recent survey in the United States reporting that over 20% of *S. pneumoniae* isolates are resistant to penicillin (14). These reports emphasize the need for new antibacterials with activity against resistant gram-positive organisms.

This study describes the PK-PD of RWJ-54428 against

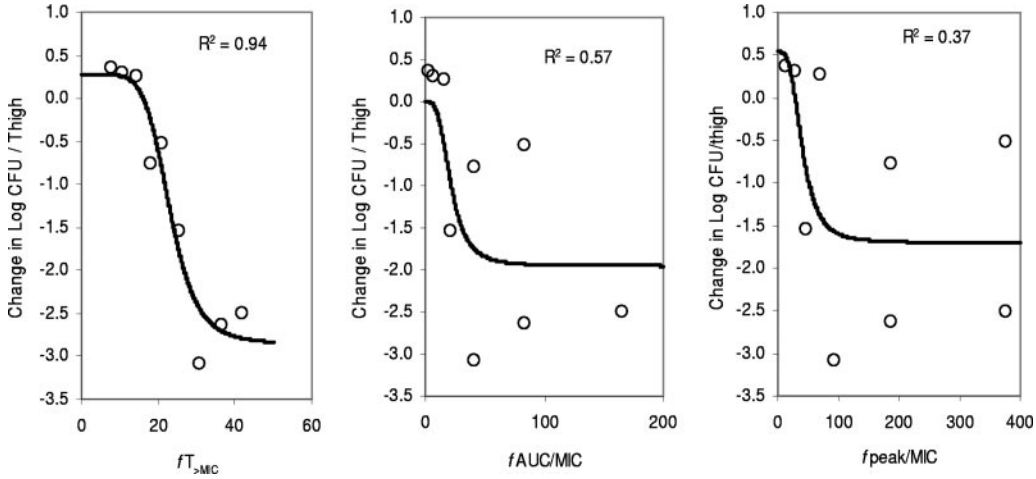

FIG. 3. Relationships between $fT_{>MIC}$, *f*AUC/MIC, and fC_{max}/MIC (*f*peak/MIC) and the change in the $\log_{10}$ number of CFU/thigh of *S. pneumoniae* SP 019. Each symbol represents the data from four thighs. The lines are the best model fits of the data. R^2 is the correlation coefficient.

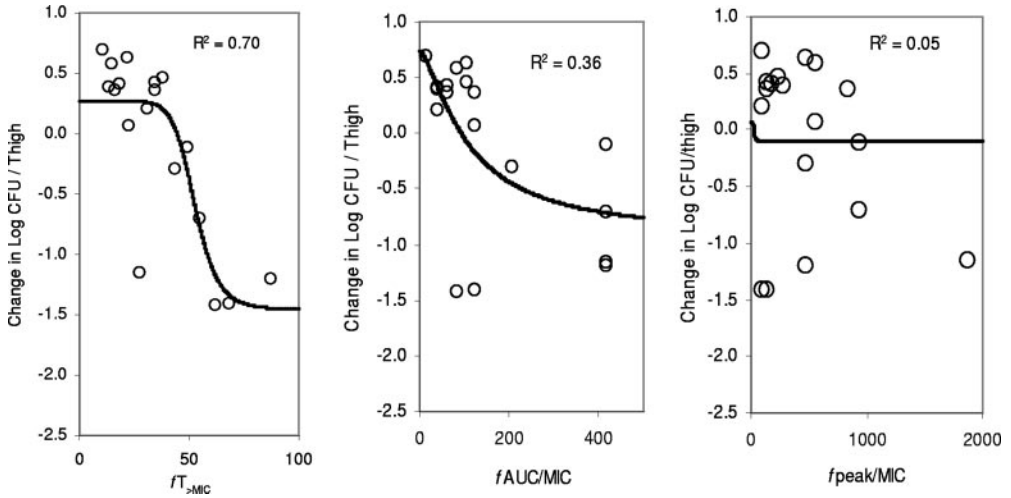

FIG. 4. Relationships between $fT_{>MIC}$, fAUC/MIC, and fC_{max}/MIC ($f$peak/MIC) and the change in the $\log_{10}$ number of CFU/thigh of *E. faecalis* EFS 007. Each symbol represents the data from four thighs. The lines are the best model fits of the data. R^2 is the correlation coefficient.

MRSA, *S. pneumoniae*, and *E. faecalis*. As was determined for other β-lactams (10, 13), $T_{>MIC}$ was the PD parameter that best correlated with the in vivo antibacterial effects of RWJ-54428. As is the case for the β-lactam class of antimicrobials, increasing concentrations did not increase the antimicrobial effect. The 24-h AUC also showed a poor correlation with efficacy, despite the presence of a prolonged in vivo postantibiotic effect against *S. aureus* (7).

Analysis of the results of the experiments with MRSA and *S. pneumoniae* revealed that a static effect (no change in the bacterial density over 24 h) is achieved with an $fT_{>MIC}$ of 14 to 20%, and a 1-log drop in bacterial density is achieved with an $fT_{>MIC}$ of 18 to 23%. These data are very similar to those for the activities of other cephalosporins against staphylococci but show a distinct difference from those for the activities of other cephalosporins against pneumococci. Craig found that cefotaxime, ceftriaxone, ceftazidime, and cefpirome achieved static effects against *S. aureus* with $fT_{>MIC}$s of 19 to 28%, but for the streptococci, these cephalosporins required $fT_{>MIC}$s of 36 to 41% (6). Analysis of the results of the experiments with *E. faecalis* showed that a static effect was not achieved until the $fT_{>MIC}$ had reached 30 to 46% and that a 1-log drop was not achieved until the $fT_{>MIC}$ reached 48 to 56%. The additional $fT_{>MIC}$ required to achieve a static effect or a 1-log drop with *E. faecalis* may be due to the slow rate of expansion observed with these organisms in this model (data not shown).

PD studies in animal models of infection have proved effective in the study of potential human dosage regimens and in the development of in vitro susceptibility breakpoints (2, 4, 5). The data for RWJ-54428 in the mouse models suggest that human dosage regimens should supply $fT_{>MIC}$s of RWJ-54428 for 20 to 30% of the interval for staphylococci and pneumococci. For enterococci, effective regimens would need $fT_{>MIC}$s of RWJ-54428 for ~50% of the dosing interval, which may be more challenging, given the MICs of many of these strains to RWJ-54428 (3).

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REFERENCES

1. **Akaike, A. C.** 1978. Posterior probabilities for choosing a regression model. Ann. Inst. Math. Stat. **30A:**9–14.
2. **Andes, D., and W. A. Craig.** 1998. In vivo activities of amoxicillin and amoxicillin-clavulanate against *Streptococcus pneumoniae*: application to breakpoint determinations. Antimicrob. Agents Chemother. **42:**2375–2379.
3. **Chamberland, S., J. Blais, M. Hoang, C. Dinh, D. Cotter, E. Bond, C. Gannon, C. Park, F. Malouin, and M. N. Dudley.** 2001. In vitro activity of RWJ-54428 (MC-02,479) against multiresistant gram-positive bacteria. Antimicrob. Agents Chemother. **45:**1422–1430.
4. **Craig, W.** 1993. Relevance of animal models for clinical treatment. Eur. J. Clin. Microbiol. Infect. Dis. **12**(Suppl. 1)**:**55–57.
5. **Craig, W. A.** 1998. Pharmacokinetics and pharmacodynamics of antibiotics in mice and men. Clin. Infect. Dis. **26:**1–12.
6. **Craig, W. A.** 1995. Interrelationship between pharmacokinetics and pharmacodynamics in determining dosage regimens for broad-spectrum cephalosporins. Diagn. Microbiol. Infect. Dis. **22:**89–96.
7. **Griffith, D. C., L. Harford, R. Williams, V. J. Lee, and M. N. Dudley.** 2003. In vivo antibacterial activity of RWJ-54428, a new cephalosporin with activity against gram-positive bactera. Antimicrob. Agents Chemother. **47:**43–47.
8. **Heffelfinger, J. D., S. F. Dowell, J. H. Jorgensen, K. P. Klugman, L. R. Mabry, D. M. Musher, J. F. Plouffe, A. Rakowsky, A. Schuchat, and C. G. Whitney.** 2000. Management of community-acquired pneumonia in the era of pneumococcal resistance: a report from the Drug-Resistant *Streptococcus pneumoniae* Therapeutic Working Group. Arch. Intern. Med. **160:**1399–1408.
9. **Herold, B. C., L. C. Immergluck, M. C. Maranam, et al.** 1998. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA **279:**593–598.
10. **Leggett, J. E., B. Fantin, S. Ebert, K. Totsuka, B. Vogelman, W. Calame, H. Mattie, and W. A. Craig.** 1989. Comparative antibiotic dose-effect relationships at several dosing intervals in murine pneumonitis and thigh-infection models. J. Infect. Dis. **159:**281–292.
11. **National Committee for Clinical Laboratory Standards.** 2004. Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard M7-A4, vol. 17, no. 2. National Committee for Clinical Laboratory Standards, Wayne, PA.
12. **Tenover, F. C.** 1999. Implications of vancomycin resistant *Staphylococcus aureus*. J. Hosp. Infect. **43**(Suppl.)**:**S3–S7.
13. **Vogelman, B., S. Gudmundsson, J. Leggett, J. Turnidge, S. Ebert, and W. A. Craig.** 1988. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. J. Infect. Dis. **158:**831–847.
14. **Whitney, C. G., M. M. Farley, J. Hadler, L.H. Harrison, C. Lexau, A. Reingold, L. Lefkowitz, P. R. Cieslak, M. Cetron, E. R. Zell, J. H. Jorgensen, and A. Schuchat.** 2000. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. N. Engl. J. Med. **343:**1917–1924.