Activities of Clindamycin, Daptomycin, Doxycycline, Linezolid, Trimethoprim-Sulfamethoxazole, and Vancomycin against Community-Associated Methicillin-Resistant *Staphylococcus aureus* with Inducible Clindamycin Resistance in Murine Thigh Infection and In Vitro Pharmacodynamic Models[▽]

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Controversy exists about the most effective treatment options for community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and about the ability of these strains to develop inducible resistance to clindamycin during therapy. Using both in vitro pharmacodynamic and murine thigh infection models, we evaluated and compared several antimicrobial compounds against CA-MRSA. Strains with inducible macrolide lincosamide-streptogramin type B (iMLS$_B$) resistance and strains in which resistance was noninducible were evaluated. Two levels of inocula (10^5 and 10^7) were evaluated for clindamycin activity in the in vivo model. In both models, the antimicrobial evaluation was performed in triplicate, and bacterial quantification occurred over 72 h, with drug doses that were designed to simulate the free drug area-under-the-concentration-time curve values (fAUCs) obtained from human samples. When the activity of clindamycin against the iMLS$_B$ strains was evaluated, constitutive resistance was noted at 24 h (MIC of >256), and failure was noted at an inoculum of $\geq 10^6$ in the in vivo models. However, at a low inoculum (10^5) in the murine thigh-infection model, clindamycin demonstrated modest activity, reducing the CFU/thigh count for clindamycin resistance-inducible strains at 72 h (0.45 to 1.3 logs). Overall, administration of daptomycin followed by vancomycin demonstrated the most significant kill against all strains in both models. Against the clindamycin noninducible strain, clindamycin and doxycycline demonstrated significant kill. Doxycycline, linezolid, and trimethoprim-sulfamethoxazide (not run in the murine model) demonstrated bacteriostatic activity against clindamycin resistance-inducible isolates. This study demonstrates that clindamycin's activity against the iMLS$_B$ strains tested is partially impacted by inoculum size. At present, there are several alternatives that appear promising for treating clindamycin resistance-inducible strains of CA-MRSA.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is established as a major cause of hospital- and community-associated infections. Most notably, MRSA infects otherwise healthy persons who have no known risk factors for a carrying drug-resistant bacteria (28). The epidemiology of community-associated MRSA (CA-MRSA) has been widely explored in several investigations; however, management of this infection has not been well studied, and it is not well established.

CA-MRSA infections can fall under a broad spectrum of infections, ranging from uncomplicated skin and soft tissue infections that can be treated in an outpatient setting to severe necrotizing fasciitis, pneumonia, and sepsis and toxic shock

syndrome, which requires hospitalization and aggressive treatment (9–11). There is renewed interest in the use of clindamycin, tetracycline, and trimethoprim-sulfamethoxazole (TMP-SMX) for treating MRSA infections as they generally have in vitro activity against CA-MRSA. For severe infections, where hospitalization and intravenous antibiotics are required, vancomycin and newer agents such as daptomycin and linezolid are feasible options.

Clindamycin remains a viable option for treating CA-MRSA, as it demonstrates in vitro susceptibility against most CA-MRSA strains. Also, when there is suspicion of streptococcus coinfection, clindamycin would be a more favorable choice than TMP-SMX, which does not have activity against streptococcus. While the clinical implications of inducible clindamycin resistance have yet to be defined, treatment failure has been reported (21, 31). Concern arises from an isolate that may possess inducible resistance that would select for constitutive resistance to clindamycin during therapy.

Using an in vitro pharmacodynamic model and a neutropenic murine thigh infection model, we evaluated the efficacy of clindamycin and several other antimicrobial agents, includ-

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ing daptomycin, doxycycline, linezolid, TMP-SMX, and vancomycin, against CA-MRSA strains, including strains that express inducible macrolide lincosamide-streptogramin type B (iMLS$_B$) resistance.

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MATERIALS AND METHODS

Bacterial strains. Clinical strains of CA-MRSA were randomly collected from a currently ongoing CA-MRSA investigation at the Detroit Medical Center (donated by William Brown, Detroit Receiving Hospital). The strains were characterized by both CDC definition of CA-MRSA and molecular typing (8, 34). We evaluated the activity of two CA-MRSA strains (R2507 and R2529) that displayed inducible iMLS$_B$ resistance, via disk diffusion testing (see below). We also evaluated antimicrobial activity against a CA-MRSA reference strain (MW2; staphylococcal chromosome cassette [SCC] *mec* type IVa) recovered from a child with fatal septicemia (9). The MW2 isolate was obtained from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program (http://www.narsa.net). MW2 is clindamycin and erythromycin susceptible (Cli^s and Ery^s, respectively).

Antimicrobial agents. Clindamycin (lot no. 109H4007), doxycycline (lot no. 110K1041), TMP-SMX (lot nos. 80K1400 and 37H1112), and vancomycin (lot no. 093K0937) were purchased from Sigma-Aldrich, St. Louis, MO. Daptomycin (lot no. CM2-282A) was provided by Cubist Pharmaceuticals, Inc., Lexington, MA. Linezolid (lot no. 03A16Z14; Pfizer) was purchased commercially. Stock solutions of each antibiotic were freshly prepared at the beginning of each week and kept frozen at −80°C.

Media. Mueller-Hinton broth (Difco Laboratories, Sparks, MD) supplemented with 25 μg/ml calcium and 12.5 μg/ml magnesium (SMHB) was used for all in vitro pharmacodynamic models. When daptomycin was evaluated, Mueller-Hinton broth was supplemented with 12.5 μg/ml magnesium and 50 μg/ml of ionized calcium according to Clinical Laboratory Standards Institute (CLSI) guidelines, due to daptomycin's dependency on physiological concentrations of calcium for its activity (7, 12, 13). Colony counts were determined using tryptic soy agar (TSA; Difco, Becton Dickinson Co., Sparks, MD) plates.

Susceptibility testing. MICs and minimum bactericidal concentrations (MBCs) of study antimicrobial agents were determined by broth microdilution with an inoculum of 5×10^5 CFU/ml in SMHB as described above, according to CLSI guidelines (12, 13). High-inoculum MIC and MBC tests were conducted as described previously (23).

Disk diffusion tests. The CLSI disk diffusion test was performed with each isolate, using unsupplemented Mueller-Hinton agar (Becton Dickinson Microbiology Systems, Cockeysville, MD) and standard 15-μg erythromycin disks and 2-μg clindamycin disks (Becton Dickinson, Sparks, MD) (12, 13). For the CA-MRSA, two disks were placed by hand to provide distances of 15 mm between the respective erythromycin-clindamycin disk pairs. Following incubation of the disks for 16 to 18 h at 35°C, zone diameters were measured. Significant ingrowth within a zone up to the edge of the disk was considered constitutive resistance. In addition, each clindamycin zone was examined carefully by using incident light to examine the plate against a dark background and by transmitted light to detect any flattening or blunting of the shape of the clindamycin zone, which indicates inducible resistance (20).

Molecular typing. Typing of the SCC *mec* elements *erm* and *mrsA* was determined by PCR amplification of the relevant regions with primers as described previously (19, 34).

In vitro pharmacodynamic model. An in vitro pharmacodynamic model consisting of a 250-ml one-compartment glass chamber with multiple ports for removal of SMHB, delivery of antibiotics, and collection of bacterial and antimicrobial samples was utilized (3). The apparatus was prefilled with medium, and antibiotics were administered as boluses infused over approximately 1 min into the central compartment via an injection port. All model simulations were conducted over 72 h and were performed in duplicate to ensure reproducibility. Prior to each experiment, several colonies from an overnight growth on TSA were added to SMHB to obtain a suspension corresponding to a 0.5 McFarland standard. Next, 2.5 ml of this suspension was added to each of the pharmacodynamic models to produce an initial starting inoculum of $10^{5.5-6.0}$ CFU/ml. Each

model was placed in a 37°C water bath for the duration of the experiment, with a magnetic stir bar to produce continuous mixing of the medium. A peristaltic pump (Masterflex model; Cole-Parmer Instrument Company, Chicago, IL) was used to continuously replace antibiotic-containing medium with fresh SMHB (at a rate that simulated the half-life [$t_{1/2}$] of the respective antibiotics). The pH was monitored throughout experiments with daptomycin due to the possible effects of pH on daptomycin activity. Free concentrations of each drug were simulated and calculated from the following total peak and trough (peak/trough) concentrations and total area-under-the-concentration-time curve (AUC) obtained from the literature: clindamycin, 600 mg every 8 h (estimated peak/trough, AUC, $t_{1/2}$, and protein binding values [percentages] were 8.3/2.1 μg/ml, 116 μg · h/ml, every 4 h, and 77%, respectively) (25); daptomycin, 6 mg/kg of body weight every 24 h (77.5/9.7 μg/ml, 468 μg · h/ml, 8 h, and 93%) (33); doxycycline, 100 mg every 12 h (10/6.6 μg/ml, 55.7 μg · h/ml, every 19.5 h, and 87%) (29); linezolid, 600 mg every 12 h (18/3.4 μg/ml, 140 μg · h/ml, every 5 h, and 31%) (22); TMP-SMX, 160-800 mg every 12 h (of 2.4/1 and 100/44 μg/ml, 48 and 875 μg · h/ml, every 10 h, and 53% and 73%, respectively); and vancomycin, 1 g every 12 h (35 to 40/11.5 μg/ml, 620 μg · h/ml, every 6 h, and 55%).

Pharmacodynamic analysis of the in vitro model. Samples (approximately 1 ml each) from each model were collected at 0, 1, 2, 4, 8, 24, 32, 48, 56, and 72 h and serially diluted in 0.9% sodium chloride. Bacterial counts were determined by plating 100-μl aliquots of each diluted sample on TSA, using an automated spiral dispenser (Whitley automatic spiral plater; Don Whitley Scientific Limited, West Yorkshire, England). All samples were diluted 10- to 100-fold before they were plated in order to minimize antibiotic carryover, and vacuum filtration was used if the final concentration was close to that of the MIC. Plated samples were incubated at 37°C for 24 h, and colony counts (log$_{10}$ CFU per milliliter) were determined using a laser colony counter (ProtoCOL software, version 2.05.02; Synbiosis, Cambridge, England). The limit of detection for this method of colony count determination is 2.0 log$_{10}$ CFU/ml. Time-kill curves were constructed by plotting mean colony counts (log$_{10}$ CFU per milliliter) from each model versus time. Bactericidal activity (99.9% kill) was defined as a ≥3-log10 CFU/ml reduction in colony count from that of the initial inoculum. Bacteriostatic activity was defined as a <3-log10 CFU/ml reduction in colony count from that of the initial inoculum, while inactive was defined as no reductions observed for the initial inoculum (23). Reductions in colony counts were determined over a 72-h period and compared between regimens.

Murine infection model. Animals were maintained in accordance with criteria of the American Association for Accreditation of Laboratory Animal Care. All animal studies were approved by the Animal Research Committee of the William S. Middleton Memorial Veterans Hospital. In each model, three 6-week-old, specific-pathogen-free, female ICR/Swiss (CD1) mice weighing 23 to 27 g were used for all studies (Harlan Sprague-Dawley, Indianapolis, IN). Three mice were used in each test group, including the control. Mice were rendered neutropenic (neutrophils, <100/mm^3) by injections of cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, IN) intraperitoneally for 4 days (150 mg/kg) and on 1 day (100 mg/kg) before thigh infection. Previous studies have shown that this regimen produces neutropenia in this model for 5 days (5). Broth cultures of freshly plated bacteria were grown to logarithmic phase overnight to an absorbance of 0.3 at 580 nm (Spectronic 88 instrument; Bausch and Lomb, Rochester, NY). After cultures were diluted 1:10 with fresh Mueller-Hinton broth, bacterial counts of the inoculum ranged from 10^5 to 10^7 CFU/thigh. Thigh infections with each of the strains were produced by the injection of 0.1 ml of inoculum into the thighs of halothane-anesthetized mice 2 h before they received therapy with each agent. In addition, 50-fold lower inocula were injected into a group of mice 2 h before they received therapy with clindamycin. After mice were inoculated, they were treated with 300 mg/kg of clindamycin every 8 h (q8h), 50 mg/kg of daptomycin every day (q.d.), 100 mg/kg of doxycycline q12h, 200 mg/kg of linezolid q12h, and 180 mg of vancomycin q12h over a 72-h period (6, 14, 30). TMP-SMX treatment was not evaluated in the mouse model because the high content of thymidine in mouse sera and tissues interferes with the activity of trimethoprim. The amount of protein binding in mouse serum is similar to that of protein binding in human serum; protein binding concentrations for mouse and human sera were as follows: clindamycin, 82% and 77%; daptomycin, 90% and 93%; doxycycline, 88% and 87%; linezolid, 30% and 31%; and vancomycin, 30% and 50%, respectively (6, 14, 30). The doses used for each of the study drugs were designed to simulate the free-drug AUC values (fAUCs) obtained with intravenous formulations of the drugs in humans (4, 18, 26, 32). Since the $t_{1/2}$s of these drugs are more rapid in mice than in humans, peak concentrations with the mice were higher than those obtained with humans. Groups of mice were sacrificed at 24 (controls), 48 (clindamycin only), and 72 h. Thighs were homogenized, serially diluted, and plated for CFU determination.

TABLE 1. Susceptibility testing results using CLSI guidelines

Antimicrobial agent	MIC (MBC) values (µg/ml) for the strain and inoculum shown					
	MW2	MW2 at high inoculum	R2507	R2507 at high inoculum	R2529	R2529 at high inoculum
Clindamycin	0.125 (0.125)	0.125 (0.25)	0.125 (0.5)	0.125 (0.5)	0.25 (4)	0.25 (1)
Daptomycin	0.063 (0.063)	0.25 (0.25)	0.06 (0.25)	0.125 (0.125)	0.125 (0.5)	0.125 (0.125)
Doxycycline	0.25 (4)	0.25 (2)	4 (16)	4 (32)	4 (16)	4 (32)
Erythromycin	0.5 (2)	0.25 (1)	64 (>64)	>64 (>64)	64 (>64)	>64 (>64)
Linezolid	2 (8)	4 (16)	2 (4)	2 (32)	2 (16)	4 (16)
TMP-SMX	0.25/5 (1/20)	0.06/1.25 (0.125/2.5)	0.5/1 (1/20)	0.06/1.25 (0.125/2.5)	0.5/10 (0.5/10)	0.125/2.5 (0.125/2.5)
Vancomycin	1 (4)	1 (1)	1 (2)	1 (1)	2 (4)	2 (4)

Antibiotic assays. For the in vitro pharmacodynamic models, samples were obtained through the injection port at 0.5, 1, 2, 4, 6, 8, 24, 28, 32, 48, 56, and 72 h for verification of target antibiotic concentrations. All samples were stored at −70°C until ready for analysis. Concentrations of vancomycin were determined using fluorescence polarization immunoassay (TDX assay; Abbott Diagnostics). The vancomycin assay has a limit of detection of 2.0 µg/ml and a between-day sample precision coefficient of variation percentage (CV%) of ≤4.8%. Linezolid, trimethoprim, and sulfamethoxazole concentrations were determined by using a validated high-performance liquid chromatography assay that conforms to the guidelines set forth by the College of American Pathologists. Linezolid sample assays were performed at the Division of Infectious Diseases at the National Jewish Medical and Research Center (Denver, CO), and trimethoprim and sulfamethoxazole sample assays were performed at the Dale E. Wurster Center for Pharmaceutical Technology, College of Pharmacy, University of Iowa. The standard curves (including the upper and lower limits of detection) ranged from 2.06 to 19.51, 0.1 to 0.5, and 8 to 20 µg/ml for linezolid, trimethoprim, and sulfamethoxazole, respectively. The between-day sample CV% values were ≤9.43% for linezolid, trimethoprim, and sulfamethoxazole. Concentrations of all other agents were determined using standard agar diffusion bioassay procedures. Each standard was tested in triplicate by placing the disks onto the appropriate agar plates, which were preswabbed with a 0.5 McFarland suspension containing the test organism. Plates were incubated for 18 to 24 h at 37°C after which time the zone sizes were measured. Clindamycin and daptomycin concentrations were determined using antibiotic assay medium 5 and *Micrococcus luteus* ATCC 9341 (2, 15). For clindamycin, the CV% was >0.95, and the intraday CV% was less than 6.25% across all standards. The lower limit of detection for the assay is 0.0625 µg/ml. For daptomycin, concentrations of 150, 100, and 10 µg/ml (1.5 µg/ml, lower limit of detection) were used as standards, with a between-day sample CV% of ≤11.1%. Doxycycline was assayed using antibiotic assay medium 8 (Difco) and *Bacillus cereus* ATCC 11778 as an indicator organism. Blank 0.25-mm disks were spotted with 20 µl of the standards or samples (1). The between-day CV% was less than 9.5% for doxycycline.

Drug assays for the in vivo murine thigh models. Plasma daptomycin samples were obtained by retroorbital puncture at 0.5, 2, 4, and 6 h from one group of infected mice and at 1, 3, 5, and 8 h from a second set of infected mice. Concentrations in plasma were determined by microbiologic assay, with *Micrococcus luteus* ATCC 9341 as the test organism. The lower limit of detection was 1.5 µg/ml. The intraday variation was less than 10% (30). Linezolid concentrations in serum were determined by high-pressure liquid chromatography analysis. Linezolid was extracted on a solid-phase cartridge and separated on a reversed-phase column with 20% (vol/vol) acetonitrile in water as the mobile phase (27). The lower limit of detection was 0.02 µg/ml. The intraday variation was <6% (6). Doxycycline, clindamycin, and vancomycin bioassays used *S. aureus* 6539p as the assay organism. The intraday variation of the assays was less than 7% for each drug. The lower limits of detection were 1.0, 0.5, and 2.0 µg/ml for doxycycline, clindamycin, and vancomycin, respectively. (6, 16, 17, 30).

Antibiotic peak and trough concentrations and $t_{1/2}$ values were calculated from concentration-time plots of the model samples, using the PKAnalyst software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT).

Detection of resistance. Samples (100 µl each) from each time point were plated onto TSA containing an antibiotic concentration of four to eight times the MIC for each organism and incubated for 48 h at 37°C to monitor for the development of resistance. Plates were visually inspected for growth of resistant subpopulations after 24, 32, and 48 h of incubation. The MIC for resistant organisms was determined using microdilution and e-testing as described above. In the in vivo studies, serial dilutions of the thigh homogenate from the clindamycin-treated mice were plated on agar containing clindamycin at 8 µg/ml.

Statistical analysis. Differences between regimens $\log_{10}$ CFU per milliliter and

$\log_{10}$ CFU per thigh at 72 h, and all pharmacodynamic variables were determined using analysis of variance with Tukey's test for multiple comparisons. For all experiments, a *P* value of ≤0.05 was considered indicative of statistical significance. All statistical analyses were performed using SPSS software (version 10.07; SPSS, Inc., Chicago, IL).

RESULTS

Susceptibility testing. Microdilution MICs and MBCs for all strains are shown in Table 1. All three organisms were susceptible to all of the drugs evaluated in the in vitro pharmacodynamic model and in the murine thigh infection model. The pattern of susceptibilities for the staphylococci tested was in accordance with expected values.

Confirmation of methicillin and erythromycin resistance. Staphylococcal strains MW2, R2507, and R2529 were positive for the *mecA* gene and were further subtyped as SCC type IV. Both of the clindamycin resistance-inducible CA-MRSA strains (R2507 and R2529) were *ermA* positive and did not express the *msrA* efflux pump gene.

Pharmacokinetics. Observed pharmacokinetic parameters (±standard deviation [SD]) for the tested agents are listed in Table 2.

Pharmacodynamics. Results of 72-h in vitro pharmacodynamic models and murine thigh models are shown in Fig. 1, 2, and 3. The magnitude of reduction in bacterial inoculum is shown in Table 3. It should be noted that positive values indicate growth.

Activity in the CA-MRSA strain in which clindamycin resistance was noninducible. In both the in vitro and the in vivo model experiments using the strain in which clindamycin resistance was noninducible (MW2), daptomycin and clindamycin demonstrated early bactericidal activity and remained bactericidal throughout 72 h (Fig. 1a). Linezolid and doxycycline demonstrated bacteriostatic activity throughout the 72-h time points, with slight regrowth noted with linezolid at 72 h. Vancomycin demonstrated an initial decrease in inoculum at 8 h, with regrowth at 24 h through 72 h, and TMP-SMX demonstrated bactericidal activity at 8 h, but regrowth to initial inoculum levels occurred after 8 h. No increase in TMP-SMX MIC was noted.

Activity of CA-MRSA strains that express iMLS$_B$ resistance. In the in vitro model, at inocula of 5.5 to 6 log10 CFU/ml (Fig. 1b and c), clindamycin demonstrated rapid kill by 8 h against the clindamycin resistance-inducible CA-MRSA strains (R2507 and R2529); however, regrowth exceeding initial inoculum levels occurred after 8 h. The initial (time zero) MICs for R2507 and R2529 were 0.5 and 0.25 µg/ml, respec-

TABLE 2. Values of free pharmacokinetic parameters targeted obtained in in vitro and in vivo models

Model	Antimicrobial agent	Regimen	fC_{max} (µg/ml) ± SD[a]	$fAUC_{0-24}$ (µg · h/ml) ± SD[b]	$t_{1/2}$ (h) ± SD
In vitro	Clindamycin	600 mg q8h	1.88 ± 0.07	21.49 ± 3.85	3.75 ± 0.07
	Daptomycin	6 mg/kg q24	7.89 ± 0.013	55.74 ± 8.39	8.11 ± 0.38
	Doxycycline	100 mg q12	1.21 ± 0.52	20.37 ± 2.13	19.1 ± 0.98
	Linezolid	600 mg q12h	11.39 ± 0.07	161.7 ± 17.14	5.47 ± 0.04
	TMP-SMX	160/800 mg q12h	1.14 ± 0.3/34.2 ± 0.57	22.70 ± 3.2/237.1 ± 2.5	10.7 ± 0.47/15.5 ± 2.0
	Vancomycin	1 g q12h	20.18 ± 2.78	258.9 ± 12.7	6.38 ± 1.17
In vivo	Clindamycin	300 mg/kg q8h		28.2	
	Daptomycin	50 mg/kg q24h		32.8	
	Doxycycline	100 mg/kg q12h		12.36	
	Linezolid	200 mg/kg q12h		198	
	TMP-SMX	ND[c]		ND[c]	
	Vancomycin	180 mg/kg q12h		272	

[a] Values shown are free (f) pharmacokinetic parameters (concentration, C, [µg/ml]) ± standard deviation (SD) obtained from in vitro and in vivo models.
[b] Free AUC values for 24 h are estimates based on the percentage of protein binding and the reported total AUC values.
[c] ND, not determined. TMP-SMX could not be evaluated in the mouse due to high thymidine concentrations in mouse tissue which interfere with TMP.

tively; however, at 24 h, the MICs for both strains exceeded 256 µg/ml, and the isolate demonstrated constitutive resistance. Daptomycin demonstrated bactericidal activity against the clindamycin resistance-inducible CA-MRSA strains as early as 2 h and remained bactericidal throughout the 72 h time points (≥3.07 log10 CFU/ml inoculum reduction). TMP-SX demonstrated rapid initial kill, but regrowth occurred after 8 h; however, resistance did not develop. Doxycycline, linezolid, and vancomycin demonstrated bacteriostatic activities against the

clindamycin resistance-inducible CA-MRSA strains, with regrowth occurring and resistance developing in the presence of doxycycline for both isolates.

Detection of resistance. Resistance was detected at 24 h when the iMLS$_B$ strains were evaluated against clindamycin in the in vitro model, whereas the MIC increased from ≤0.5 to >256 µg/ml. Also, the clindamycin resistance-inducible strains demonstrated a twofold increase in MIC from baseline against doxycycline at 48, 56, and 72 h.
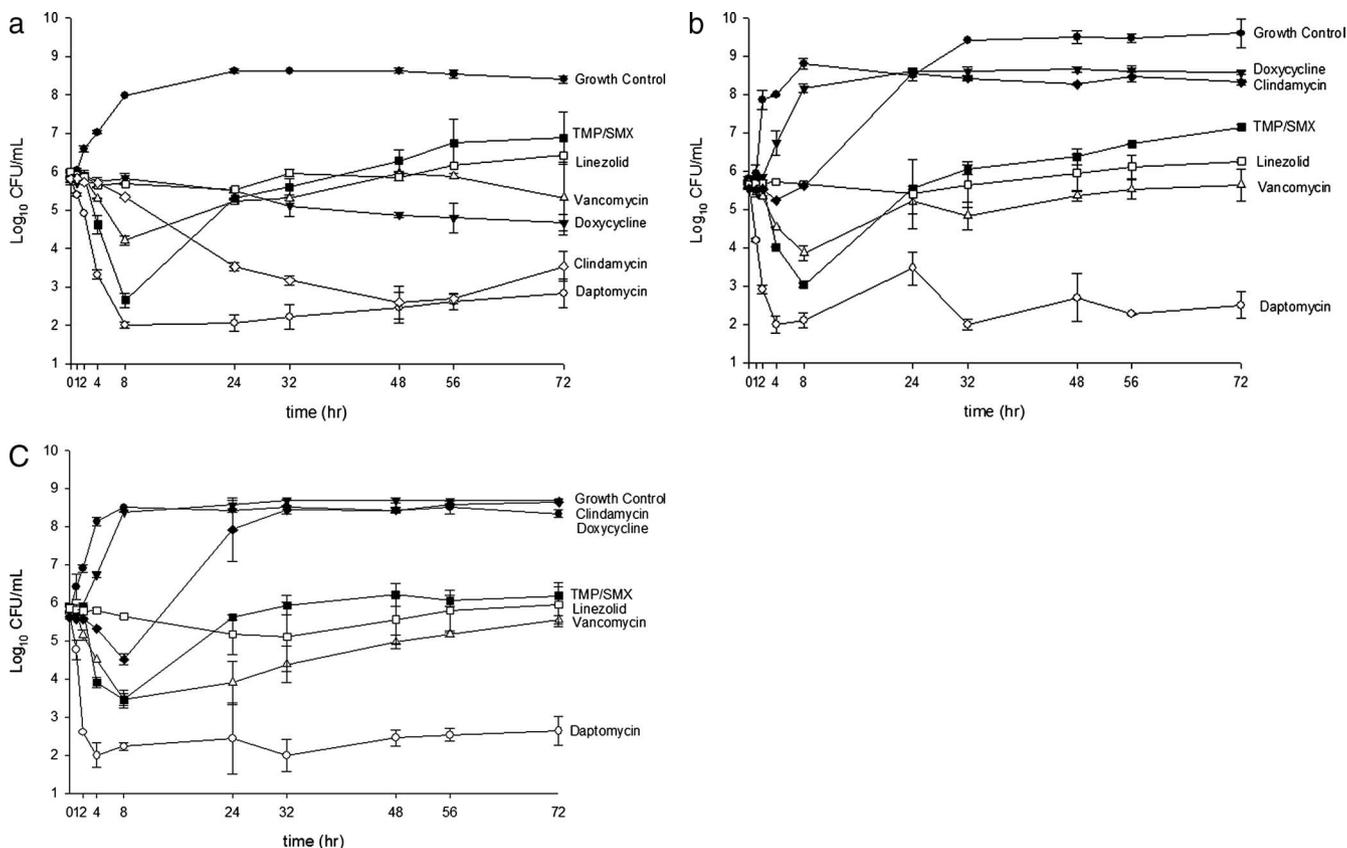

FIG. 1. Activities of the antimicrobials tested in the in vitro model against a strain in which clindamycin resistance is noninducible (MW2) (a) and clindamycin resistance-inducible strains R2507 (b) and R2529 (c).
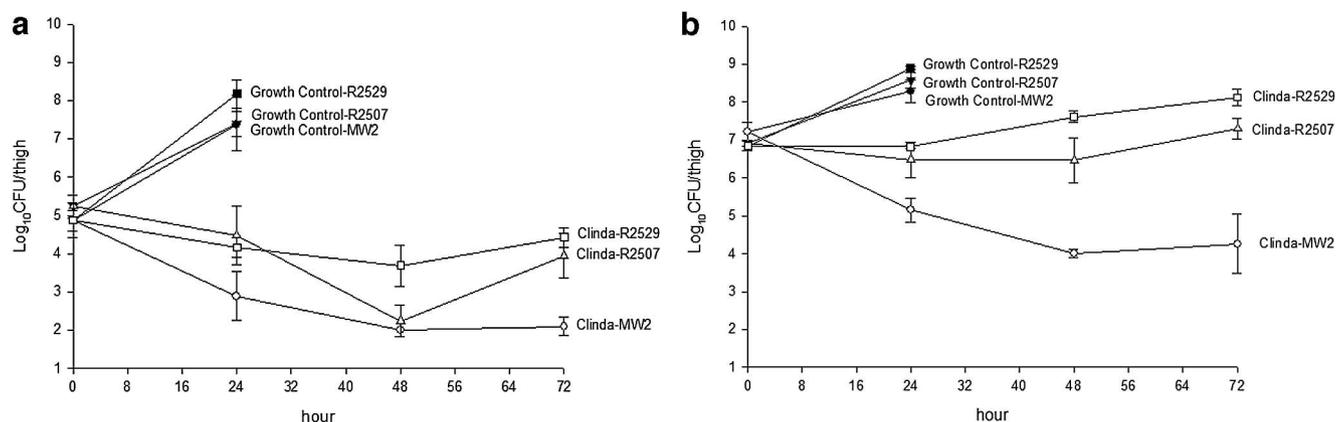
FIG. 2. Means (±SD) log10 CFU/thigh at 24, 48, and 72 h following therapy with clindamycin against CA-MRSA strains MW2, R2507, and R2529 at (a) low and (b) high inocula are shown. Note that after 24 h, all mice in the control group were sacrificed.

Murine infection model. The mean ± SD log10 CFU/thigh values at 24, 48, and 72 h following therapy with clindamycin low and higher inocula are shown in Fig. 2 and 3. At 24 h, all mice in the control group were sacrificed. At inocula of $10^{6.8–7.2}$ CFU/thigh, clindamycin reduced the CFU/thigh of only the strain in which clindamycin resistance was noninducible at 24 h (2.1 logs) and 72 h (3.0 logs), respectively. In experiments evaluating the clindamycin resistance-inducible strains, clindamycin demonstrated bacteriostatic activity at 24 h, and the isolate displayed modest growth (0.39 and 1.28 logs, respectively) at 72 h. However, at lower inocula ($10^{4.9–5.2}$ CFU/thigh), clindamycin reduced the CFU/thigh for all three strains at 24 h (0.5 to 0.8 logs) and 72 h (0.5 to 1.3 logs), respectively. Resistant colonies were detected at 24 h with the high inoculum and at 72 h with the low inoculum. The mean ± SD log10 CFU/thigh values at 24 h and 72 h following therapy with daptomycin, doxycycline, linezolid, and vancomycin are shown in Fig. 3.

At the high inoculum, daptomycin demonstrated significant killing activity at 24 (−2.4 to −3.5 logs) and 72 h (−3.6 to −4.1 logs) against all three strains, while linezolid (−0.9 to +0.5 logs) and vancomycin (−0.8 to −0.1 logs) were primarily bacteriostatic. Doxycycline resulted in 1.2 and 2.1 logs of killing at

24 and 72 h, respectively, with the strain in which clindamycin resistance was noninducible but was ineffective (0.6 to 0.7 log growth at 24 h) with swollen (appeared infected) thighs for both clindamycin resistance-inducible strains.

A comparison of the changes in CFU/thigh at 72 h with those obtained from the in vitro model over the same time period are shown in Table 3. At the higher inoculum, clindamycin was more active against the strain in which clindamycin resistance was noninducible and resulted in only modest growth with the two clindamycin resistance-inducible strains. Doxycycline was less active in the in vivo model against the two clindamycin resistance-inducible strains, as the MICs were 4 μg/ml. Daptomycin, linezolid, and vancomycin exhibited relatively similar activity in both the in vivo and the in vitro models.

DISCUSSION

In the advent of increased MRSA skin infections in the community and the need for effective oral out-patient therapies, clindamycin, TMP-SMX, doxycycline, and potentially linezolid remain practical treatment options for CA-MRSA. Additionally, as more complicated infections by these CA-MRSA
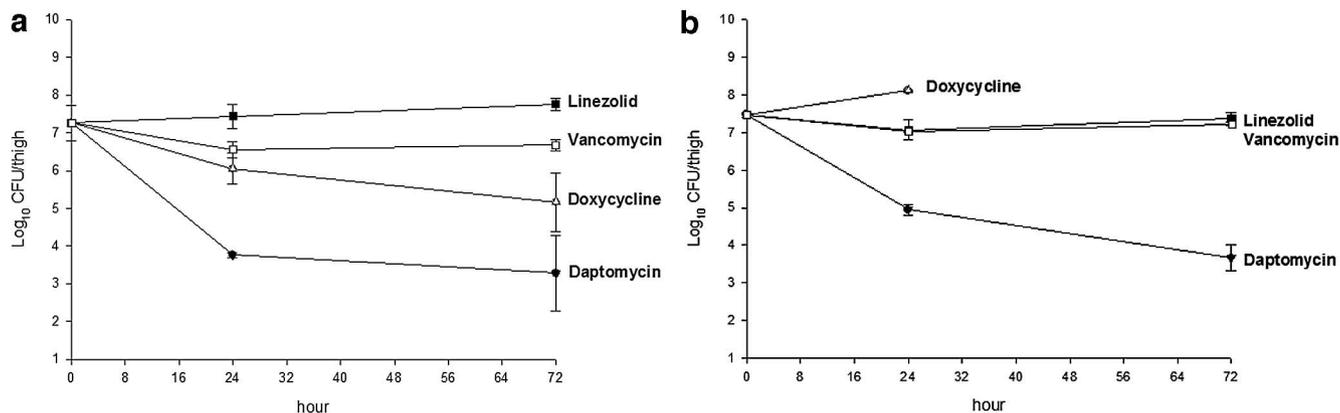

FIG. 3. Time course of the antimicrobial activity of multiple antibiotics against CA-MRSA (a) MW2 and (b) R2507 and R2529 average. Means (±SD) log10 CFU/thigh at 24 and 72 h following therapy with daptomycin, doxycycline, linezolid, and vancomycin at the higher inoculum are shown. Note that after 24 h, the mice in the control and doxycycline groups were sick and all were sacrificed, per the American Association for Accreditation of Laboratory Animal Care protocol. All other mice were sacrificed at 72 h.

TABLE 3. Comparison of in vitro and in vivo pharmacodynamic changes[a]

Organism	Drug[a]	Change in log10 ± SD	
		In vitro (CFU/ml)	In vivo (CFU/g)
MW2	Clindamycin (HI)	−2.27 ± 0.21[b]	−2.96 ± 0.40[b]
	Clindamycin (LI)		−2.77 ± 0.64
	Daptomycin	−2.94 ± 0.24[b]	−3.98 ± 0.54[b]
	Doxycycline	−1.09 ± 0.09	−2.09 ± 0.59[b]
	Linezolid	+0.43 ± 0.03	+0.5 ± 0.24
	TMP-SMX	+1.07 ± 0.48	
	Vancomycin	−0.65 ± 0.61	−0.58 ± 0.18
R2507	Clindamycin (HI)	+2.79 ± 0.04[b]	+0.39 ± 0.44[b]
	Clindamycin (LI)		−1.3 ± 0.59
	Daptomycin	−3.17 ± 0.22[b]	−4.06 ± 0.35[b]
	Doxycycline	+2.83 ± 0.01[b]	+0.62 ± 0.34 (at 24 h)
	Linezolid	+0.60 ± 0.06	−0.4 ± 0.38
	TMP-SMX	+1.49 ± 0.04	
	Vancomycin	−0.29 ± 0.24	−0.44 ± 0.29
R2529	Clindamycin (HI)	+3.03 ± 0.02[b]	+1.28 ± 0.14[b]
	Clindamycin (LI)		−0.45 ± 0.35
	Daptomycin	−3.07 ± 0.26[b]	−3.56 ± 0.45[b]
	Doxycycline	+2.83 ± 0.05[b]	+0.66 ± 0.14 (at 24 h)
	Linezolid	+0.11 ± 0.35	−0.2 ± 0.08
	TMP-SMX	+0.33 ± 0.09	ND
	Vancomycin	−0.29 ± 0.03	−0.07 ± 0.25

[a] Data compare the changes in CFU/ml or CFU/g (thigh) at 72 h with those obtained from the in vitro pharmacokinetic model over the same time period. HI, high inoculum (>6 log10 CFU/ml or CFU/g); LI, low inoculum (<5 log10 CFU/ml or CFU/g). ND, not done.

[b] P values of <0.05, representing a significant difference from the kill of the 72-h growth control.

strains are seen, the intravenous therapies with agents such as vancomycin, daptomycin, and linezolid will be used. When selecting appropriate treatment, it is imperative to weigh the risks and benefits of each agent prior to prescribing. It is therefore important that the treating clinician is aware that inducible resistance may occur during treatment with clindamycin (21, 22). Studies indicate that inducible resistance to clindamycin can occur in approximately 13.3% of Cli^s and Ery^r CA-MRSA strains and 36% to 56% of all hospital-associated MRSA strains (24, 31) These frequencies emphasize the importance of additional laboratory methods for evaluating inducible clindamycin resistance prior to using clindamycin for the treatment of MRSA (12, 13).

Our study evaluated the activity of CA-MRSA strains with both inducible clindamycin resistance and noninducible clindamycin resistant properties. We discovered that against an *S. aureus* inoculum of $\geq 10^6$ CFU/ml in a clindamycin resistance-inducible strain, clindamycin was ineffective and demonstrated constitutive resistance as early as 24 h. This occurred in both the in vitro and the in vivo models. However, when a lower inoculum was used in the in vivo model, clindamycin demonstrated modest activity against the clindamycin resistance-inducible strains. These findings suggest that a relationship between the in vivo inoculum constitutive clindamycin resistance in *S. aureus* may exist. This may help explain some of the variability seen in clinical results described in the literature with clindamycin therapy with similar strains (31).

Although the peak concentrations used in the in vitro model

may be higher than those drug concentrations observed with patients, the order of potency and the extent (fAUC) of activity were quite similar in the in vitro and the in vivo models. In both models, daptomycin demonstrated the most activity, and linezolid, doxycycline, and vancomycin demonstrated bacteriostatic activity. TMP-SMX was tested only in the in vitro model but has been shown to have potential for treating skin and soft tissue infections caused by CA-MRSA.

With regard to comparing the results between the in vitro and in vivo models, clindamycin was most effective in the thigh infection model at a low inoculum. In addition, the emergence of constitutive clindamycin resistance appeared to be delayed in the in vivo model. Doxycycline displayed similar nonsignificant activity in the thigh infection and the in vitro model. Daptomycin was bactericidal in both models, while linezolid and vancomycin were primarily bacteriostatic. Although our in vitro and in vivo results demonstrated consistency among all regimens, our results should be applied to clinical practice with caution, and the confirmation of our results with clinical studies would be beneficial before these regimens can be adopted for use in the care of patients.

In conclusion, the activity of all compounds for treating the CA-MRSA strain in which clindamycin resistance is noninducible is daptomycin > clindamycin > doxycycline > vancomycin = linezolid = TMP-SMX; in the clindamycin inducible (iMLSb) strains, daptomycin > vancomycin = linezolid > clindamycin. Clindamycin was not effective at high inocula against the two clindamycin inducible strains tested.

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