In Vitro and In Vivo Antistaphylococcal Activities of L-695,256, a Carbapenem with High Affinity for the Penicillin-Binding Protein PBP 2a

HENRY F. CHAMBERS*

The Medical Service, San Francisco General Hospital, and The University of California, San Francisco, San Francisco, California

L-695,256 is a synthetic carbapenem β-lactam antibiotic that binds with a high degree of affinity to penicillin-binding protein (PBP) PBP 2a, the protein that mediates staphylococcal resistance to methicillin. The concentration of L-695,256 that inhibited binding of radiolabeled [^{3}H]penicillin to PBP 2a by 50% was 1.2 µg/ml, whereas they were 14 and 68 µg/ml for penicillin and imipenem, respectively. Cell wall synthesis, determined by incorporation of [^{14}C]N-acetylglucosamine into whole cells, was inhibited by 50% at concentrations of 1.3, 26, and 132 µg/ml for L-695,256, penicillin, and imipenem, respectively, for the methicillin-resistant strain COL. Growth of cells of each of two homogeneously resistant strains, COL and 76, was completely inhibited by 4 µg of L-695,256 per ml, whereas growth was inhibited by 100 µg or more of penicillin or imipenem per ml. The efficacies of L-695,256 (10 mg/kg given three times daily [t.i.d.]), imipenem (37.5 mg/kg t.i.d.), penicillin (300,000 units/kg t.i.d.), and vancomycin (25 mg/kg given twice daily) were compared in the rabbit model of aortic valve endocarditis established with these homogeneous strains. After 4 days of treatment, mean bacterial densities in aortic valve vegetations were reduced by 4.0 to 5.8 log$_{10}$ CFU/g for L-695,256, 1.0 to 1.8 log$_{10}$ CFU/g for imipenem, −1.1 to 3.9 log$_{10}$ CFU/g for penicillin, and 1.1 to 3.0 log$_{10}$ CFU/g for vancomycin in comparison to the densities of controls. Compounds such as L-695,256 that are bound by PBP 2a with a high degree of affinity are likely to be extremely effective in the treatment of infections caused by methicillin-resistant staphylococci.

The role of β-lactam antibiotics in the treatment of infections caused by methicillin-resistant staphylococci has been controversial. Activity in vitro typically does not indicate efficacy in vivo, mainly because of the characteristically heterogeneous expression of methicillin resistance such that only a very small proportion of cells, e.g., 1 in 10^4 to 10^6 cells, expresses the trait. When high-inoculum infections are treated with β-lactam antibiotics, susceptible cells are eradicated, but resistant cells survive and continue to grow, leading to therapeutic failure (2, 4).

Methicillin resistance in staphylococci is mediated by a low-affinity penicillin-binding protein (PBP), PBP 2a. The concentration at which a β-lactam antibiotic binds to PBP 2a closely corresponds to its concentration that inhibits the growth of the most resistant subpopulation of cells within a culture (3). In comparison with other β-lactam antibiotics, penicillin, ampicillin, and amoxicillin are bound by PBP 2a with a relatively high degree of affinity (3, 5). When they are administered in very large doses (e.g., 600 to 800 mg of ampicillin or amoxicillin per kg of body weight per day) to achieve concentrations in serum exceeding those needed to bind PBP 2a, ampicillin and amoxicillin have been shown to be active in animal models of endocarditis caused by methicillin-resistant strains (1, 5, 10). Unfortunately, the large doses that were required are at the limits of those which are achievable clinically (14), and a β-lactamase inhibitor also must be administered to prevent hydrolysis of ampicillin or amoxicillin by staphylococcal β-lactamase. Nevertheless, these in vitro and in vivo experiments indicate

that if β-lactam antibiotics with a high degree of affinity for binding PBP 2a were available they very likely would be effective.

The purpose of the present studies was to characterize the antibacterial activity of an investigational carbapenem, L-695,256, against methicillin-resistant strains of *Staphylococcus aureus*. L-695,256 is unique among β-lactam antibiotics because it is bound by PBP 2a with a high degree of affinity and it is β-lactamase stable (7; this work). The antibacterial activity of L-695,256 against methicillin-resistant staphylococci in vitro was determined, and the concentrations that inhibited growth were compared with those that bound PBP 2a and inhibited cell wall synthesis. The efficacy of L-695,256 also was examined in the rabbit model of aortic valve endocarditis caused by homogeneous, highly methicillin-resistant strains of *S. aureus.*

MATERIALS AND METHODS

Antibiotics. L-695,256 was provided by Merck Sharpe & Dohme Research Laboratories (Rahway, N.J.). Its chemical structure is shown in Fig. 1. The other antibiotics were obtained from the hospital pharmacy of San Francisco General Hospital.

Bacterial strains. *S. aureus* COL, COL-8a, and 76 were used for the endocarditis studies. COL is a β-lactamase-negative, homogeneously resistant strain (8). Strain 76 is a β-lactamase-producing homogeneous strain (15). COL-8a is a methicillin-susceptible spontaneous mutant of COL obtained by passage at 43°C. It produces no PBP 2a detectable by fluorography or in immunoblots with anti-PBP 2a polyclonal rabbit antiserum (6). DNA sequence analysis (19) of the *mecA* gene of COL-8a revealed a guanine insertion mutation at nucleotide 325, resulting in a frameshift with a stop codon at the position of amino acid residue 115 and the termination of translation of mRNA into complete protein.

Strains 27S (8) and 209P (both β-lactamase-negative susceptible strains), 67-O (β-lactamase positive and negative variants, heterogeneously resistant) (2, 9), and 27R (β-lactamase-negative, homogeneously resistant transformant of 27S) (8) were used for in vitro studies and in PBP-binding assays.

Susceptibility studies. MICs were determined by a standard microdilution method in cation-supplemented Mueller-Hinton broth with 2.0% NaCl (NaCl

* Mailing address: Division of Infectious Diseases, San Francisco General Hospital, Box 0811, 3rd and Parnassus Avenue, University of California San Francisco, San Francisco, CA 94143. Phone: (415) 206-8666. Fax: (415) 206-6015.
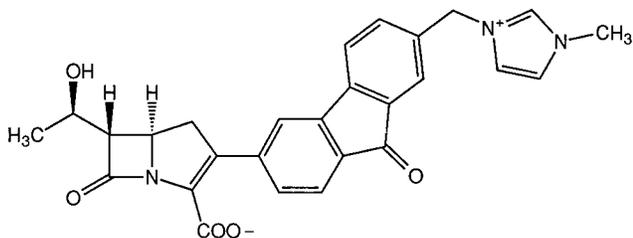
FIG. 1. Chemical structure of L-695,256, (5R,6S)-2-[7-(1-methylimidazolium-3-yl)methyl-9-fluorenone-3-yl]-6-(1R-hydroxyethyl)-carbapen-2-em-3-carboxylate.

was omitted for vancomycin) at an inoculum of approximately 3×10^5 to 5×10^5 CFU/ml. MICs were read after 24 h at 35°C.

Population analysis was performed by quantitative inoculation of overnight culture in tryptic soy broth onto Trypticase soy agar containing concentrations of drug ranging from 0 to 1,000 µg/ml. Cultures were incubated for 48 h at 37°C, at which time the colonies were counted.

PBP-binding studies. Binding of β-lactam antibiotics to PBPs was determined by fluorography of membrane proteins labeled in a competition assay (3). Exponentially growing cells were harvested at a cell density of 2×10^8 to 5×10^8 CFU/ml, washed, and then mechanically disrupted. Membranes were separated by differential centrifugation and were resuspended in 10 mM Tris-HCl (pH 7.0) to a final protein concentration of 10 mg/ml assayed by the Bradford method. Samples of 10 µg of membrane protein were preincubated with either buffer (direct PBP assay) or antibiotic at each of several concentrations up to 1,000 µg/ml (competition assay) for 15 min at 37°C. A total of 20 µg of [³H]penicillin per ml was then added for 15 min. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were stained with Coomassie blue strain. Binding of antibiotics to PBPs was quantified by scanning densitometry of the fluorographs. Binding was expressed as the concentration of nonradiolabeled antibiotic that inhibited radiolabeling with [³H]penicillin by 50% (IC_{50}) compared with that of a control sample.

Cell wall incorporation studies. Exponentially growing cells in TSB were back diluted into equal volumes of tryptic soy broth and were radiolabeled in a 30-min incubation at 37°C with [¹⁴C]N-acetylglucosamine (¹⁴C-NAG) in the presence of drug at a final concentration of 0, 0.1, 1, 10, 100, or 1,000 µg/ml. Cells were harvested, washed to remove unincorporated radiolabel, and lysed by incubation in lysostaphin (100 mg/ml) at 37°C for 30 min. The disintegrations per minute incorporated into cells from equal volumes of culture unexposed and exposed to drug were determined by liquid scintillation counting. The percentage of disintegrations per minute of drug-exposed cells at each drug concentration relative to those of unexposed cells was determined for each drug concentration. The IC_{50}, the concentration at which incorporated counts were reduced to 50% of that in unexposed cells, was determined by linear regression.

Rabbit endocarditis model. The concentrations of penicillin, imipenem, and L-695,256 were determined in serum samples obtained from groups of three uninfected rabbits administered a single dose of each drug. Concentrations were assayed by the agar well diffusion method (18). The concentrations in serum and the half-lives of vancomycin and sulbactam were determined previously (4).

To establish endocarditis a catheter was positioned across the aortic valve and was left in place (16). Twenty-four hours later 1 ml of approximately 10^7 CFU of the infecting organism in 0.9% NaCl was injected intravenously. The first dose of antibiotic was administered 18 to 24 h later (see Table 1 for regimens). Treatment was given for 4 days. The data for rabbits that died within the first 24 h of therapy were not included in the data analysis. For rabbits that died after 24 h,

the vegetations were removed and processed. Data for these rabbits were included in the data analysis. Surviving rabbits were sacrificed approximately 12 h after administration of the last dose. Aortic valve vegetations were removed aseptically, homogenized in 0.5 ml of 0.9% saline, and quantitatively subcultured onto blood agar to determine the total number of organisms remaining in the vegetation. This number, the vegetation titer, was expressed as $\log_{10}$ CFU per gram of vegetation. The lower limit of detection was 1 $\log_{10}$ CFU/g; sterile vegetations were assigned a value of 0. Differences between mean vegetation titers of the several groups were tested for statistical significance (defined as $P < 0.05$) by the t test with the Bonferoni correction for multiple comparisons.

RESULTS

Susceptibility studies. The MICs of L-695,256 and the other β-lactam antibiotics for methicillin-susceptible strains of *S. aureus* were similar, but L-695,256 was 32 to 64 times more active by weight against highly methicillin-resistant strains (Table 2). L-695,256 was approximately 50 to 100 times more potent by weight than penicillin or imipenem in inhibiting growth, as determined by population analysis (Table 3).

PBP binding. Results of PBP-binding assays are shown in Table 4. PBPs 1, 2, 3, and 4 bound L-695,256 at IC_{50}s similar to those observed for penicillin and imipenem. PBP 2a bound L-695,256 at concentrations that were 10 to 50 times lower than those of penicillin and imipenem. Binding of L-695,256 to PBP 2a was similar in each of the three methicillin-resistant strains, COL, 27R, and 67-O, that were examined.

Cell wall incorporation studies. The IC_{50}s of penicillin, imipenem, and L-695,256 were 0.1, 0.1, and 0.05 µg/ml, respectively, for COL-8a in ¹⁴C-NAG incorporation assays. These values correlated well with the MICs, which were also less than or equal to 0.1 µg/ml, and with the IC_{50}s of 0.05 to 0.2 µg/ml for PBPs 1, 2, and 3, the PBPs that mediate the antibacterial effects of β-lactam antibiotics in susceptible strains of staphylococci (17). The IC_{50}s of penicillin, imipenem, and L-695,256 for COL in cell wall incorporation assays were 26, 132, and 1.3

TABLE 2. MICs of study drugs

Drug	MIC (µg/ml) for the following strains (phenotype)[a]:				
	209P (sus)	COL-8a (sus)	COL (hom)	67-O (het)[b]	76 (hom)
Penicillin	≤0.1	≤0.1	32	16	ND[c]
Imipenem	≤0.1	≤0.1	32	4	64
Vancomycin	ND	4	2	1	1
L-695,256	≤0.1	≤0.1	0.5	2	2

[a] sus, methicillin susceptible; hom, homogeneous, methicillin resistant; het, heterogeneous, methicillin resistant.
[b] β-Lactamase-negative variant of strain 67-O.
[c] ND, not done.

TABLE 1. Pharmacokinetics for single doses of antibiotics used for endocarditis studies

Drug	Dose, interval[a]	Concn (µg/ml) in serum at 1 h	Half-life (min)
Penicillin[b]	300,000 units/kg, t.i.d.	38 ± 8 ($n = 3$)	190
Imipenem	37.5 mg/kg, t.i.d.	16 ± 4 ($n = 3$)	28
L-695,256	10 mg/kg, t.i.d.	21 ± 8 ($n = 3$)	52
Vancomycin[c]	25 mg/kg, b.i.d.	43 ± 7 ($n = 6$)	78
Sulbactam[c]	50 mg/kg, t.i.d.	40 ± 14 ($n = 9$)	29

[a] t.i.d., three times daily; b.i.d., twice daily.
[b] Penicillin was administered as 150,000 U of penicillin G plus 150,000 U of procaine penicillin.
[c] Concentrations in serum and half-lives for vancomycin and sulbactam were determined previously (4).

TABLE 3. Population analysis with concentrations that inhibited 99 and 100% of CFU in an agar plating assay of *S. aureus* strains used in the endocarditis studies

Drug	Concn (µg/ml) that inhibited the following strains by the indicated amount:					
	COL-8a		COL		76	
	99%	100%	99%	100%	99%	100%
Penicillin	0.1	0.5	50	100	20[a]	200
Imipenem	0.1	5	50	500	ND[b]	ND
L-695,256	0.1	1	1	4	1	4

[a] The β-lactamase inhibitor sulbactam was included at a sulbactam:penicillin ratio of 1:2 by weight.
[b] ND, not done.

TABLE 4. Mean IC_{50} of antibiotics for binding of *S. aureus* PBPs[a]

Drug	Mean IC_{50} (μg/ml [range]) for binding of PBPs:				
	1	2	2a	3	4
Penicillin	0.2 (0.1–0.4)	0.05 (0.03–0.1)	14 (11–17)	0.03 (0.01–0.04)	6 (2–13)
Imipenem	0.05 (0.03–0.07)	0.08 (0.07–0.09)	68 (55–90)	0.05 (0.03–0.07)	0.02 (0.02)
L-695,256	0.1 (0.1–0.2)	0.2 (0.1–0.2)	1.2 (0.4–1.8)	0.1 (0.02–0.1)	0.3 (0.1–0.7)

[a] The mean IC_{50}s for PBPs 1, 2, 3, and 4 for each drug were determined by averaging the four values obtained for strains 209P, COL-8a, 67-O, and 27R. The mean values for PBP 2a for each drug were determined by averaging the three values obtained for strains COL, 67-O, and 27R.

μg/ml, respectively and correlated with the IC_{50}s for PBP 2a, but not the other PBPs, consistent with the role of PBP 2a as the critical target of β-lactam antibiotics in resistant cells. L-695,256 was 20 and 100 times more potent than penicillin and imipenem, respectively.

Endocarditis studies. The concentrations in serum and the half-lives of the antibiotics used in the treatment of endocarditis are given in Table 1. The pharmacokinetics of penicillin and vancomycin were such that these drugs were present at therapeutic concentrations for longer periods than L-695,256. At a dose of 300,000 U/kg, serum penicillin concentrations would be expected to exceed the IC_{50} for PBP 2a for 3 to 4 h after dosing (Fig. 2).

All β-lactam antibiotic regimens were effective against the susceptible strain, COL-8a, with an approximately 3- to 4-$\log_{10}$ CFU/g reduction in vegetation bacterial counts over the 4 days of therapy (Table 5). Ten rabbits were treated with a lower dose of penicillin, 100,000 U/kg three times daily. This lower dose of penicillin was also effective, with mean bacterial counts of 4.9 ± 1.8 $\log_{10}$ CFU/g in rabbits treated for 4 days. Vancomycin was relatively ineffective, producing only a 1.1-$\log_{10}$ CFU/g reduction in bacterial density in vegetations compared with that in the group that received no treatment.

L-695,256 and penicillin were the most effective agents against the resistant strain, COL (Table 5). Imipenem was not effective, nor was vancomycin, which reduced the counts by only 1.1 $\log_{10}$ CFU/g over the 4 days of therapy, the same outcome that was observed for the susceptible strain, COL-8a. Separate experiments were performed to assess the effect of lowering the dose of penicillin to 100,000 U/kg three times daily. On the basis of the concentrations in serum produced by the 300,000-U/kg dose, reducing this dose by two-thirds would

be expected to produce concentrations in serum at or below those needed to bind PBP 2a. This lower dose of penicillin was less effective in reducing bacterial counts in vegetations; rabbits given 100,000 units/kg three times daily had a bacterial density of 6.2 ± 2.0 ($n = 9$) $\log_{10}$ CFU/g, compared with 3.3 ± 1.9 ($n = 8$) for rabbits given 300,000 U/kg three times daily ($P = 0.008$).

A homogeneous, β-lactamase-producing methicillin-resistant strain, strain 76, was also examined in the endocarditis model (Table 6). As with the COL strains, L-695,256 was the most effective drug; this was followed by vancomycin and then imipenem. Penicillin, even though it was administered with 50 mg of the β-lactamase inhibitor sulbactam per kg, was ineffective.

DISCUSSION

The activities of β-lactam antibiotics against phenotypically resistant cells present in methicillin-resistant strains of *S. aureus*, whether homogeneous or heterogeneous strains, are closely related to the concentration at which PBP 2a is bound (3). Of the β-lactam antibiotics currently available for use, imipenem, penicillin, ampicillin, and amoxicillin are bound by PBP 2a with a relatively high degree of affinity and are the most active drugs in vitro. When administered in large doses that achieve concentrations in serum at which binding of PBP 2a occurs, these antibiotics show some activity in experimental animal models of endocarditis caused by methicillin-resistant staphylococci (1, 5, 10). These results are consistent with the view that PBP 2a is the critical target in methicillin-resistant strains and demonstrate that successful therapy of methicillin-resistant staphylococcal infections with β-lactam antibiotics is
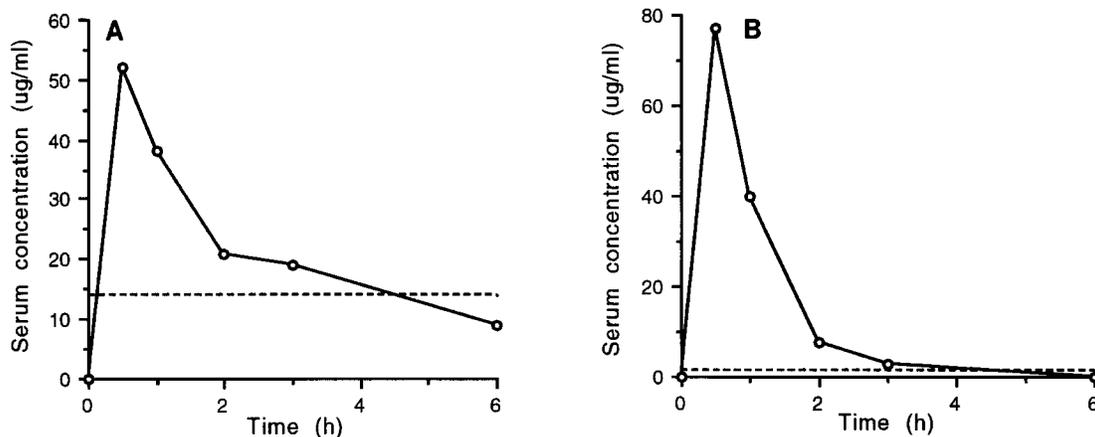

FIG. 2. Concentrations in serum over time of penicillin (A) achieved after intramuscular injection of 300,000 U/kg (150,000 U each of penicillin G and procaine penicillin) and of L-695,256 (B) achieved after intramuscular injection of 10 mg/kg, each with respect to the IC_{50} (in micrograms per milliliter, indicated by the broken line) of the drug for PBP 2a.

TABLE 5. Vegetation titers of bacteria, number of animals with sterile vegetations, and deaths for endocarditis studies with the COL-8a and COL strain pair[a]

Regimen	COL-8a				COL			
	No. of deaths at:		No. of sterile vegetations	Vegetation titer ($\log_{10}$ CFU/g)[b]	No. of deaths		No. of sterile vegetations	Vegetation titer ($\log_{10}$ CFU/g)
	<24 h	≥24 h			<24 h	≥24 h		
No treatment			0	9.3 ± 0.5 (8)			0	7.8 ± 1.4 (10)
Vancomycin	2	1	0	8.2 ± 0.9 (9)	1	2	1	6.7 ± 2.7 (15)
Imipenem	0	2	0	5.6 ± 1.8 (11)[c,d]	1	1	1	6.0 ± 3.0 (14)
Penicillin	0	1	0	6.6 ± 1.0 (8)[c]	0	1	0	3.9 ± 1.3 (13)[c,d]
L-695,256	1	0	0	5.6 ± 1.4 (8)[c,d]	1	1	0	3.8 ± 2.2 (14)[c,d]

[a] Mean values do not include rabbits that died within the first 24 h of therapy.
[b] Values are means ± standard deviations. Values in parentheses indicate numbers of rabbits that had vegetation titers determined.
[c] $P < 0.05$ compared with no treatment.
[d] $P < 0.05$ compared with vancomycin.

at least theoretically possible. Unfortunately, the very large doses of the drugs that are required are a serious limitation and may not be practical clinically.

L-695,256, an investigational β-lactam antibiotic, was examined as a prototype drug that binds PBP 2a at a concentration that is in the range likely to be therapeutically relevant, i.e., less than 10 μg/ml. L-695,256 was 10 to 100 times more potent in vitro against methicillin-resistant staphylococci than the most active β-lactam antibiotics currently available. The activity of L-695,256 against methicillin-resistant staphylococci was associated with its high-affinity binding to PBP 2a. The results of cell wall incorporation assays indicated that the inhibitory effect of L-695,256 was apparently due to its specific binding of PBP 2a. L-695,256 was approximately 10 times more potent than penicillin in inhibiting growth, binding to PBP 2a, and inhibiting cell wall synthesis.

The activity of L-695,256 that was observed in vitro was also evident in vivo. L-695,256 was the most effective agent for the treatment of endocarditis caused by either of two highly and homogeneously methicillin-resistant strains. Penicillin was effective for endocarditis caused by the β-lactamase-negative COL strain at a dose approximately 20 times that of L-695,256 (300,000 U/kg, equivalent to 190 mg of penicillin per kg, versus 10 mg of L-695,256 per kg every 8 h). Efficacy was associated with concentrations of L-695,256 and penicillin in serum that were sufficient to bind PBP 2a for a period of approximately 4 h after dosing (Fig. 2). Efficacy was lost at the lower dose of penicillin for the resistant COL strain, but not its susceptible variant, COL-8a. These data strongly suggest that the activity

of L-695,256 was due to its binding of PBP 2a and that β-lactam antibiotics which are bound by PBP 2a with a high affinity would be effective against methicillin-resistant *S. aureus* infections.

Vancomycin is the only drug to which all strains of *S. aureus*, including methicillin-resistant strains, are susceptible, and it is the drug of choice for treating infections caused by methicillin-resistant staphylococci. The relatively recent identification of vancomycin-resistant strains of enterococci (11) and the potential for transfer of this resistance to staphylococci (13) increase the urgency for finding effective alternatives to vancomycin for the treatment of staphylococcal infections. Moreover, a growing body of clinical (12, 20) and experimental (1, 5) data suggest that vancomycin is intrinsically less active (e.g., less rapidly bactericidal) than β-lactam antibiotics against *S. aureus*. The lack of efficacy of vancomycin for endocarditis caused by the COL and COL-8a strains supports this conclusion. These are additional reasons to pursue the development of β-lactam antibiotics which are active against methicillin-resistant staphylococci.

ACKNOWLEDGMENTS

TABLE 6. Vegetation titers, number of rabbits with sterile vegetations, and deaths for endocarditis studies with strain 76[a]

Regimen	No. of deaths		No. of sterile vegetations	Vegetation titer[b]
	<24 h	≥24 h		
No treatment			0	7.8 ± 0.9 (9)
Vancomycin	0	0	0	4.8 ± 2.4 (13)[c]
Imipenem	0	6	0	6.8 ± 2.1 (12)[d]
Penicillin[e]	0	3	0	8.9 ± 0.4 (7)[d,f]
L-695,256	0	0	4	2.0 ± 2.1 (12)[c,f]

[a] Mean values do not include rabbits that died within the first 24 h of therapy.
[b] Values are means ± standard deviations. Values in parentheses indicate numbers of rabbits that had vegetation titers determined.
[c] $P < 0.05$ compared with no treatment.
[d] $P < 0.05$ compared with L-695,256.
[e] Sulbactam (50 mg/kg) was coadministered with each dose of penicillin.
[f] $P < 0.05$ compared with vancomycin.

REFERENCES

1. **Cantoni, L., A. Wenger, M. Glauser, and J. Bille.** 1989. Comparative efficacy of amoxicillin-clavulanate, cloxacillin, and vancomycin against methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* in rats. J. Infect. Dis. **159:**989–993.
2. **Chambers, H. F., C. J. Hackbarth, T. A. Drake, M. G. Rusnak, and M. A. Sande.** 1984. Methicillin-resistant *Staphylococcus aureus* endocarditis in rabbits: expression of resistance in vivo and in vitro. J. Infect. Dis. **149:**894–903.
3. **Chambers, H. F., and M. Sachdeva.** 1990. Binding of beta-lactam antibiotics to penicillin-binding proteins in methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. **161:**1170–1176.
4. **Chambers, H. F., M. Sachdeva, and S. Kennedy.** 1990. Binding affinity for penicillin-binding protein 2a correlates with in vivo activity of beta-lactam antibiotics against methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. **162:**705–710.
5. **Franciolli, M., J. Bille, M. P. Glauser, and P. Moreillon.** 1991. Beta-lactam resistance mechanisms of methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. **163:**514–523.
6. **Gerberding, J. L., C. Miick, H. H. Liu, and H. F. Chambers.** 1991. Comparison of conventional susceptibility tests with direct detection of penicillin-binding protein 2a in borderline oxacillin-resistant strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **35:**2574–2579.
7. **Hammond, G. G., K. M. Overbye, and L. L. Silver.** 1994. Binding of L-695,256, a prototypical 2-aryl carbapenem antibiotic, to penicillin binding proteins of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus hirae*, abstr. F62, p. 127. *In* Program and abstracts of the 34th Inter-

science Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

8. **Hartman, B. J., and A. Tomasz.** 1984. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J. Bacteriol. **158:**513–516.

9. **Hartman, B. J., and A. Tomasz.** 1986. Expression of methicillin resistance in heterogeneous strains of *Staphylococcus aureus*. Antimicrob. Agents Chemother. **29:**85–92.

10. **Hirano, L., and A. S. Bayer.** 1991. β-lactam–β-lactamase-inhibitor combinations are active in experimental endocarditis caused by β-lactamase-producing oxacillin-resistant staphylococci. Antimicrob. Agents Chemother. **35:**685–690.

11. **LeClerq, R., E. Derlot, J. Duval, and P. Courvalin.** 1988. Plasmid mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319:**157–161.

12. **Levine, D. P., B. S. Fromm, and B. R. Reddy.** 1991. Slow response to vancomycin or vancomycin plus rifampin in methicillin-resistant *Staphylococcus aureus* endocarditis. Ann. Intern. Med. **115:**674–680.

13. **Noble, W. C., Z. Virani, and R. G. Cree.** 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS Microbiol. Lett. **72:**195–198.

14. **Norris, S., C. H. Nightingale, and G. L. Mandell.** 1990. Tables of antimicrobial agent pharmacology, p. 434–460. *In* G. L. Mandell, R. G. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases, 3rd ed. Churchill Livingstone, New York.

15. **Peacock, J. E., F. J. Marsik, and R. P. Wenzel.** 1980. Methicillin-resistant *Staphylococcus aureus*: introduction and spread within a hospital. Ann. Intern. Med. **93:**526–532.

16. **Perlman, B. B., and L. R. Freedman.** 1971. Experimental endocarditis. II. Staphylococcal infection of the aortic valve following placement of a polyethylene catheter in the left side of the heart. Yale J. Biol. Med. **44:**206–213.

17. **Reynolds, P. E.** 1988. The essential nature of staphylococcal penicillin-binding proteins, p. 343–351. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Shockman (ed.), Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.

18. **Sabath, L. D., and J. P. Anhalt.** 1980. Assay of antimicrobics, p. 485–490. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.

19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.

20. **Small, P. M., and H. F. Chambers.** 1990. Vancomycin for *Staphylococcus aureus* endocarditis in intravenous drug users. Antimicrob. Agents Chemother. **34:**1227–1231.