MAJOR ARTICLE

Impact of Antibiotics on Expression of Virulence-Associated Exotoxin Genes in Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus*

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Extracellular protein toxins contribute to the pathogenesis of a wide variety of *Staphylococcus aureus* infections. The present study investigated the effects that cell-wall active antibiotics and protein-synthesis inhibitors have on transcription and translation of genes for Panton-Valentine leukocidin, alpha-hemolysin, and toxic-shock syndrome toxin 1, in both methicillin-sensitive and methicillin-resistant *S. aureus*. Subinhibitory concentrations of nafcillin induced and prolonged mRNA for Panton-Valentine leukocidin, alpha-toxin, and toxic-shock syndrome toxin 1 and increased toxin production. In contrast, clindamycin and linezolid markedly suppressed translation, but not transcription, of toxin genes. These results suggest (1) that protein-synthesis inhibition is an important consideration in the selection of antimicrobial agents to treat serious infections caused by toxin-producing gram-positive pathogens and (2) that, by inducing and enhancing toxin production, inadvertent use of beta-lactam antibiotics to treat methicillin-resistant *S. aureus* infections may contribute to worse outcomes.

There is clear evidence that alpha-hemolysin plays a significant role in local tissue necrosis in animal models of staphylococcal infection [1]. In addition, toxic-shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxin B (SEB) play important roles in menstruation- and surgery-associated staphylococcal toxic-shock syndrome (STSS), respectively (reviewed in [2]). Recently, an epidemic of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) infections has emerged throughout the United States. These strains have an SCC*mec* type IV cassette conferring resistance to methicillin [3], and 77% of them harbor genes for Panton-Valentine leukocidin (PVL) [4, 5]. In addition, the prevalence of the genes for alpha-toxin and SEB is higher in CA-MRSA than in hospital-acquired MRSA (HA-MRSA), suggesting that strains circulating in the community are more virulent than hospital-associated strains [6]. Recent reports of necrotizing pneumonia and necrotizing fasciitis caused by CA-MRSA strains harboring PVL genes support this conclusion [7–9]. In addition, STSS caused by MRSA strains producing SEB, staphylococcal enterotoxin (SEC), or TSST-1 has been reported from many parts of the world [3, 10–15]. Remarkably, in a few short years, MRSA has spread globally, causing more severe infections, which are due, in part, to an increase in their toxin armamentarium.

The effect on toxin production is an important consideration in the selection of an antimicrobial agent to treat staphylococcal infections. Despite the emergence of MRSA, nafcillin remains a potentially valuable antibiotic for methicillin-sensitive *S. aureus* (MSSA) infections since beta-lactam antibiotics are bactericidal by blocking penicillin-binding protein (PBP)–mediated cell-wall synthesis. Yet, at concentrations at or above the MIC, nafcillin actually increases TSST-1 concen-

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trations in culture supernatant fluid, likely because of rupture of the cell wall and release of toxin into the external milieu [16]. At subinhibitory concentrations, nafcillin increases alpha-toxin production via induction of mRNA production [17]. The present study compared the effects of nafcillin and vancomycin versus those of 2 protein-synthesis inhibitors, clindamycin and linezolid, on production of alpha-toxin, TSST-1, and PVL and on the temporal expression of toxin-specific mRNA in both MSSA and MRSA.

MATERIAL AND METHODS

Strains and culture conditions. Several strains of MSSA and MRSA were used in the present study (table 1). MSSA strain 04-002 was isolated from the abdominal wound of a patient with STSS and was found to produce high levels of TSST-1 [18]. The other clinical isolate, strain 04-014, was obtained from the Centers for Disease Control and Prevention (CDC strain 368-04). This strain was isolated from a case of CA-MRSA infection and produces SEC, TSST-1, and alpha-hemolysin but is negative for PVL. No multilocus sequence typing or *agr* analysis was done on this strain. Pulse-field gel electrophoresis (PFGE) analysis was performed but did not yield a match (within 80%) to any known USA type; it was most closely related to strains of the USA 600 type (R. Carey, personal communication). The MSSA laboratory strain—Wood 46, which produces high levels of alpha-hemolysin—was purchased from the American Type Culture Collection VA (ATCC strain 18032). Last, MRSA strain 1560 was a provided by Dr. Francoise Perdreau-Remington, University of California, San Francisco [19]. This CA-MRSA strain was isolated from a wound and belongs to ST1 (USA 400); its PFGE pattern is similar, but not identical, to that of MW2; and it is PVL positive and contains SCC*mec* IV and *agr* III (table 1).

All strains of staphylococcus were maintained in cation-supplemented Mueller-Hinton II broth. When the effects that antibiotics have on alpha-hemolysin, TSST-1, and PVL production were examined, *S. aureus* was cultured in beef-heart infusion (BHI) medium supplemented with 16.5 mmol/L glucose, 25 mmol/L $NaHCO_3$, 34 mmol/L NaCl, 6 mmol/L Na_2HPO_4, 1.35 mmol/L L-glutamine, and 20 mmol/L Mg_2SO_4 (complete BHI [cBHI]). This medium supports maximal TSST-1 production [20–23] and permits alpha-hemolysin and PVL production. To initiate cultures, single bacterial colonies were picked from sheep-blood/agar plates, and cultures were grown at 37°C in 5% CO_2 with shaking (100 rpm).

Antibiotics. The antibiotics used in the present study were nafcillin (Bristol-Meyers-Squibb), vancomycin (Sigma), linezolid (Pfizer), and clindamycin HCl (Pfizer). MICs of these antibiotics were determined by a microdilution broth method used according to NCCLS guidelines [24], except that cBHI medium was used instead of cation-supplemented Mueller-Hinton broth; these MICs are provided in table 1.

Antibiotics' effects on toxin production. Experiments designed to test the effects that antibiotics have on TSST-1, alpha-hemolysin, and PVL production required that high starting concentrations of inocula be used to obtain detectable levels of toxin; specifically, cultures were initiated with washed *S. aureus* at 5×10^7 cfu/mL and were allowed to grow for ~4 h (mid-log phase), at which point, designated as time 0, antibiotics were added. To compensate for the increased inoculum size, vancomycin, linezolid, and clindamycin were added such that the final concentration was 5 times the MIC; this concentration was chosen on the basis of our previous work on antibiotic-induced toxin suppression in *Clostridium perfringens* [25] and group A streptococcus (authors' unpublished data). Nafcillin was used at a concentration of 0.01–8 μg/mL. Samples were removed at time 0 and at various times after antibiotic administration and were placed on ice. The number of viable bacteria at each time point was determined by plating duplicate 10-μL samples (or 10-fold dilutions thereof) onto blood/agar plates. The remaining sample was rendered bacteria free by centrifugation and filter sterilization and then was frozen at

Table 1. Characteristics of strains used in the present study.

Strain	Response to methicillin	*mecA* type	*agr* type	MIC, μg/mL				Toxin profile (by gene probe)		
				Nafcillin	Vancomycin	Clindamycin	Linezolid	PVL	TSST-1	Alpha-hemolysin
04-014	Resistant	IVc	ND	6.4	S[a]	S[a]	S[a]	−	+	+
MRSA 1560	Resistant	IV	III	6.4	2.0	0.2	2.0	+	−	+
04-002	Sensitive	NA	ND	0.8	1.0	0.1	4.0	−	+	+
ATCC 18032	Sensitive	NA	ND	1.6	ND	ND	ND	−	−	+
ATCC 29213[b]	Sensitive	NA	ND	0.4	2.0	0.1	4.0	ND	ND	ND

NOTE. NA, not applicable; ND, not done; PVL, Panton-Valentine leukocidin; TSST-1, toxic-shock syndrome toxin 1; −, negative; +, positive.

[a] Antibiotic sensitivity, verified by disk diffusion in the Veterans Administration Medical Center Clinical Microbiology Laboratory.

[b] Control strain of *Staphylococcus aureus*. The MICs shown were obtained in our laboratory and are within the defined ranges for this strain.

Table 2. Primers used in the present study.

Gene	Oligonucleotide	
	Sense	Antisense
tst	GCTACAGATTTTACCCCTGTTCCCTTA	TGATATGTGGATCCGTCATTCATTG
hla	CCAATCGATTTTATATCTTTCTGAAGAACG	ATTGCTAGGTTCCATATTAATGAATCCTGT
lukS	GATAACAATATTGAGAAT	AAGTGAAAGGACATAATTGA

−70°C until it was tested, as described below, for the presence of toxins.

Toxin assays. The TSST-1 ELISA followed procedures described elsewhere [26]. In brief, a 96-well microplate was coated, overnight at 4°C, with 10 μg/mL rabbit polyclonal anti–TSST-1 (Toxin Technology) in Dulbecco's PBS (DPBS), after which the wells were washed and blocked with 3% bovine serum albumin in DPBS. Either bacterial culture–supernatant fluid or recombinant TSST-1 (Toxin Technology) containing 1% normal rabbit serum to block nonspecific binding of antibodies by protein A was added, in duplicate, to the plate, followed by rabbit anti–TSST-1 horseradish-peroxidase conjugate (diluted 1:300) (Toxin Technology) and the colorimetric substrate, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Zymed). After 20 min, absorbance was read at 405 nm. The concentration of TSST-1 in the culture sample was extrapolated, by linear regression, from a standard curve prepared by use of recombinant TSST-1. The assay was linear over the range of 1.0–62.5 ng/mL, with 0.5 ng/mL as the lower limit of detection.

Alpha-hemolysin activity was measured by hemolysis of rabbit erythrocytes, as described elsewhere [1, 17, 27]. In brief, sterile filtered culture-supernatant fluid was diluted 2-fold in DPBS in a microtiter plate, and an equal volume of a washed, rabbit-erythrocyte suspension (2% in DPBS) was added. Sterile deionized water served as the 100% hemolysis control. After 1 h at 37°C, the plate was centrifuged, the supernatant removed to a fresh microtiter plate, and absorbance was read at 550 nm. Activity, in hemolytic units per milliliter, was defined as the inverse of the dilution causing 50% hemolysis, multiplied by 2.

The presence of PVL was determined by western blotting using a leukocidin F (LukF)–PV–specific polyclonal rabbit antiserum that was prepared as follows: A 27-aa peptide—RYTNFWNQLHWIGNNYKDENRATHTSI—corresponding to aa 275–301 of mature LukF-PV, was selected for anti-PVL antibody production because this region is the most divergent from the F-components of the other homologous bicomponent toxins (i.e., LukF, gamma-hemolysin component B, and LukE) in *S. aureus* and is not present in the S-components (reviewed in [28]). Antipeptide antibody was commercially prepared (Phoenix Phamaceuticals) by repeated immunization of rabbits with a KHL-peptide conjugate, and titers were followed by western blotting of recombinant LukF-PV. The LukF-PV peptide antiserum recognized recombinant LukF-PV but showed no cross-reactivity, by western blot, either with recombinant LukS-PV or with culture-supernatant fluid from strain ATCC 31889, which produces LukF and gamma-hemolysin but is LukF-PV negative [28] (data not shown).

Northern blotting. Approximately 1×10^9 *S. aureus* were collected by centrifugation at 13,000 *g* in a microfuge tube. Total RNA was purified by use of a RiboPure-Bacteria kit (Ambion), according to the manufacturer's instructions. The RNA yield and its quality were assessed by UV absorbance and by agarose-gel electrophoresis, respectively. Two micrograms of total RNA in an ~4-μL volume was mixed with 10 μL of RNA loading buffer (catalog number R-4268; Sigma), and the sample was heated at 55°C for 15 min. The sample was loaded onto a 1% denaturing agarose gel and was run under 100 V for 90 min. RNA was transferred, by capillary action, to a nylon membrane (Hybond-N; Amersham Life Science) by use of 20× standard saline citrate (SSC) buffer. The membrane was air-dried, baked at 80°C for 2 h, and prehybridized in Ultrahyb solution (Ambion) at 42°C for 1 h, followed by hybridization with random labeled (Invitrogen) ^{32}P probes (described below), at a concentration of 1×10^6 counts/mL, for 18 h at 42°C. The membrane was washed twice, at 60°C for 15 min, with 2× SSC and 1% SDS and twice, at 60°C for 15 min, with 0.2× SSC and 0.5% SDS and then was exposed to Kodak BioMax light film (Sigma).

Gene-specific probes for TSST-1, alpha-hemolysin, and the S-component of PVL were prepared by polymerase chain reaction (PCR) amplification and random labeling of the oligonucleotides listed in table 2. The resultant PCR products cover the TSST-1 and Hla molecules from aa 72–629 and aa 25–933, respectively. The PVL primers amplified the entire LukS-PV gene.

RESULTS

Antibiotics' effects of on growth and PVL mRNA expression in MRSA. Addition of nafcillin, vancomycin, clindamycin, or linezolid during the mid-log phase had different effects on the growth of MRSA strain 1560 (figure 1*A*). Specifically, low (0.2 or 2 μg/mL) doses of nafcillin had little effect on growth, such that by 10 h there was no difference between the bacterial concentration in these cultures and that in untreated control cultures. In contrast, both linezolid and clindamycin imme-

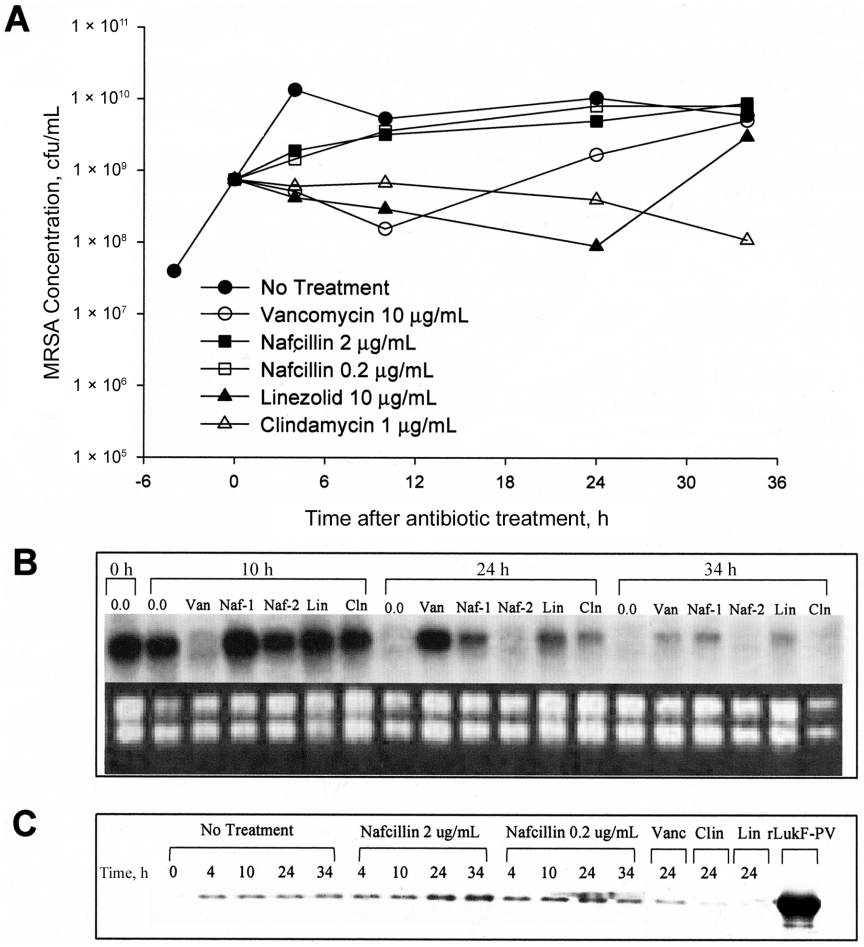

Figure 1. Effect of antibiotics on growth and PVL production in methicillin-resistant *Staphylococcus aureus* (MRSA) strain 1560. Antibiotics were added, at the final concentrations indicated, at the mid-log phase of growth; this time is designated Time 0. Samples were obtained at Time 0 and at various times up to 34 h after antibiotic treatment for quantitation of MRSA *(A),* detection of PVL mRNA by northern blotting *(B),* and detection of PVL protein by western blotting *(C),* as described in Materials and Methods. The panel beneath the northern blot shows ethidium bromide–stained RNA that was transferred to the nylon membrane and photographed to illustrate that equivalent amounts of RNA were loaded per lane. Naf-1 and Naf-2 in the northern blot *(B)* denote nafcillin at 2.0 and 0.2 μg/mL, respectively. Data shown are from 1 representative experiment of 2 that were performed with this community-acquired MRSA strain. Data for MRSA concentration represent the means of duplicate plate counts at each time point; the discrepancy between replicates is too small to be visible. Cln, clindamycin; Lin, linezolid; Naf, nafcillin; rLukF-PV, recombinant Panton-Valentine leukocidin F component.

diately inhibited growth: in clindamycin-treated cultures, bacterial viability remained static for 24 h, after which time a slow killing ensued; linezolid was slowly bacteriocidal from 4 to 24 h, after which time growth resumed such that the bacterial concentration at 34 h was only slightly less than that in untreated control cultures. Vancomycin decreased bacterial viability by 10 h, after which time near log-phase growth resumed.

mRNA for PVL was strongly expressed in mid-log–phase cultures before the addition of antibiotics (i.e., time 0 h) (figure 1*B*). In untreated control cultures, production of PVL mRNA continued at 10 h but disappeared by 24 h. In contrast, vancomycin decreased PVL mRNA expression by 10 h. However, when this culture resumed near-log growth (at 10–24 h), PVL mRNA was again strongly expressed; by 34 h, mRNA expression remained detectable although significantly reduced. At 10 h, the amount of PVL mRNA in the nafcillin-, clindamycin-, and linezolid-treated cultures was not significantly different from that in the untreated control culture; however, by 24 h, when mRNA was no longer apparent in the untreated control culture, it remained significantly expressed in the vancomycin-treated culture and modestly expressed in the linezolid-, clindamycin-, and 2-μg/mL–nafcillin-treated cultures. By 34 h, mRNA in these cultures had waned but was still discernible in all but the untreated control culture.

Production of PVL in untreated control cultures was measurable by western blot as bacteria approached stationary phase (at 4 h) (figure 1*C*), with little additional toxin accumulation in the supernatant thereafter. In contrast, clindamycin and li-

nezolid markedly suppressed PVL production such that no toxin was detectable at times up to 12 h after antibiotic administration (not shown). Furthermore, the maximal amount of PVL that had accumulated by 24 h in the clindamycin- and linezolid-treated cultures was barely discernible by western blotting (figure 1*C*). Suppression of PVL production occurred despite the prolongation of mRNA expression induced by these 2 antibiotics. In contrast, nafcillin at 2 μg/mL stimulated continued toxin production beyond 10 h, as evidenced by stronger bands on the western blot at 24 and 34 h. Last, the level of PVL production in vancomycin-treated cultures was comparable to that in the untreated control cultures and was higher than that in clindamycin- or linezolid-treated cultures.

Nafcillin-induced changes in* S. aureus *TSST-1 and alpha-hemolysin gene expression. The results of the studies using MRSA strain 1560 (figure 1) suggested that nafcillin increased toxin production by inducing and prolonging toxin-gene transcription. To determine whether this was a general phenomenon, nafcillin-induced mRNA expression and toxin production were similarly evaluated in 3 additional strains of *S. aureus* producing TSST-1 and/or alpha-hemolysin.

In untreated MRSA and MSSA cultures, TSST-1 mRNA peaked during the postexponential phase of growth (i.e., 10–12 h) and virtually disappeared during the stationary phase (24 h) (figure 2*A* and 2*B, lower panels*). In contrast, nafcillin at a concentration of 2, 4, or 8 μg/mL prolonged TSST-1 mRNA expression into the stationary phase (24 h), both in the MRSA strain (i.e., strain 04-014) (figure 2*A*) and the MSSA clinical strain (i.e., strain 04-002) (figure 2*B*). Nafcillin at a concentration of 0.2 μg/mL also sustained TSST-1 mRNA in the MRSA strain (figure 2*A*). The nafcillin-induced prolongation of gene transcription was independent of the antibiotic's effects (or lack thereof) on bacterial growth (figure 2*A* and 2*B, upper panels*).

Similarly, nafcillin prolonged the expression of alpha-hemolysin mRNA in both the MRSA strain (i.e., strain 04-014) (figure 3*A*) and the MSSA laboratory strain (i.e., ATCC strain 18032) (figure 3*B*). Specifically, in the MRSA strain, nafcillin concentrations from 0.2 to 8 μg/mL prolonged alpha-hemolysin mRNA expression; the greatest effect was observed with the highest concentration of the antibiotic (figure 3*A*). In contrast, for the MSSA laboratory strain, a bell-shaped relationship was observed, in that the mid-level concentration (i.e., 0.2 μg/mL) of nafcillin was most effective at up-regulating (at 4 and 12 h) and prolonging (at 24 h) alpha-hemolysin mRNA (figure 3*B*).

Nafcillin's effect on production of TSST-1 and alpha-hemolysin. In all cases, up-regulation of mRNA expression by nafcillin was associated with increased and prolonged toxin production, irrespective of the specific strain studied. Specifically, in both MRSA and MSSA, TSST-1 production was increased at 24 h (figure 4*A*) and continued to increase at 34 h in the MRSA strain (data not shown). Similarly, enhanced alpha-hemolysin production at 24 h was induced by low-dose (0.2 μg/mL) nafcillin, in both the MRSA strain and the MSSA laboratory strain (figure 4*B*). Interestingly, for the MSSA laboratory strain, the 0.2-μg/mL dose of nafcillin also both induced the greatest mRNA expression and was the optimal concentration for its prolongation (figure 3, *lower panel*). For both strains, nafcillin concentrations ⩾2 μg/mL resulted in reduced alpha-hemolysin production (figure 4*B*), despite prolonged mRNA expression at these concentrations, which suggests that saturation of the presumed target PBPs play an as-yet-undefined role in either synthesis regulation or toxin release from the cell.

Interestingly, for the MRSA strain 04-014, the nafcillin concentration associated with the greatest TSST-1 production was 10-fold higher (i.e., 2 μg/mL) than that required for optimal alpha-hemolysin production (i.e., 0.2 μg/mL) in this strain. This discrepancy occurred despite the fact that mRNA for the 2 toxins was equally prolonged by nafcillin (compare figures 2 and 3). It is also of interest that, for all strains of *S. aureus,* high concentrations of nafcillin decreased toxin production despite prolongation of mRNA expression at 24 h.

DISCUSSION

Treatment of *S. aureus* infections changed dramatically with the emergence of methicillin resistance among strains causing hospital-associated infections (i.e., HA-MRSA strains). More recently, clones of MRSA (e.g., USA300 and USA400) have emerged in the community [9, 29, 30], and these CA-MRSA are responsible for severe infections, including toxic shock, necrotizing fasciitis, and necrotizing pneumonia [7, 9]. These strains have a unique *mecA* cassette (type IV) [3, 9, 31] and, at present, have greater antibiotic susceptibility than do the HA-MRSA strains [29]. Compared with the HA-MRSA strains, CA-MRSA strains also have a higher prevalence of toxin genes, including the enterotoxins, TSST-1, and PVL [32]. PVL causes tissue necrosis in experimental animals [33] and has been implicated, at least epidemiologically, in severe infections in humans, such as necrotizing fasciitis and necrotizing pneumonia [7, 8, 19, 34]; at present, however, no data exist that define a role for PVL in the pathogenesis of CA-MRSA infections.

We and others have shown that suppression of toxin production improves outcomes in animal models and human cases of severe necrotizing infections caused by group A streptococcus and clostridial species [35–37]. Given the strong association between toxin-producing CA-MRSA and severe necrotizing infections in humans, such agents may also prove beneficial in the treatment of these infections. Indeed, the present study demonstrates that both clindamycin and linezolid suppress PVL production in CA-MRSA. Similarly, others have demonstrated that protein-synthesis inhibitors suppress virulence factors of MSSA (e.g., TSST-1, alpha-hemolysin, and coagulase) [16, 38–

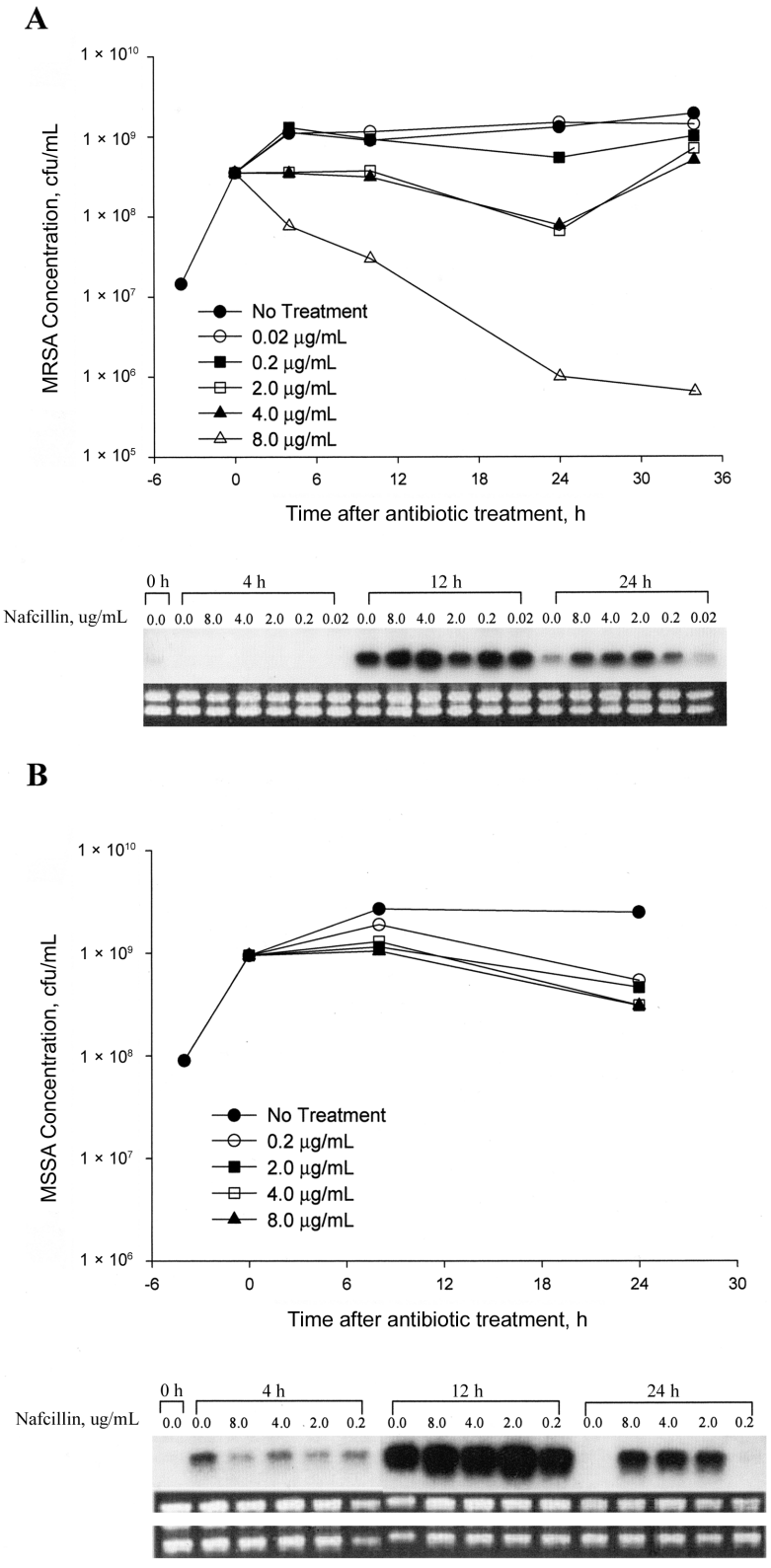

Figure 2. Effects of nafcillin on growth and toxic-shock syndrome toxin 1 (TSST-1) mRNA expression in methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) strains. Nafcillin was added at the indicated concentrations at the mid-log phase of growth (time 0), and samples were taken at various times for bacterial quantitation and analysis of TSST-1 gene expression by northern blotting. Data shown are from 1 representative experiment of 3 for MRSA strain 04-014 *(A)* and of 4 for MSSA strain 04-002 *(B)*. Data for MRSA and MSSA concentrations represent the means of duplicate plate counts at each time point; the discrepancy between replicates is too small to be visible.

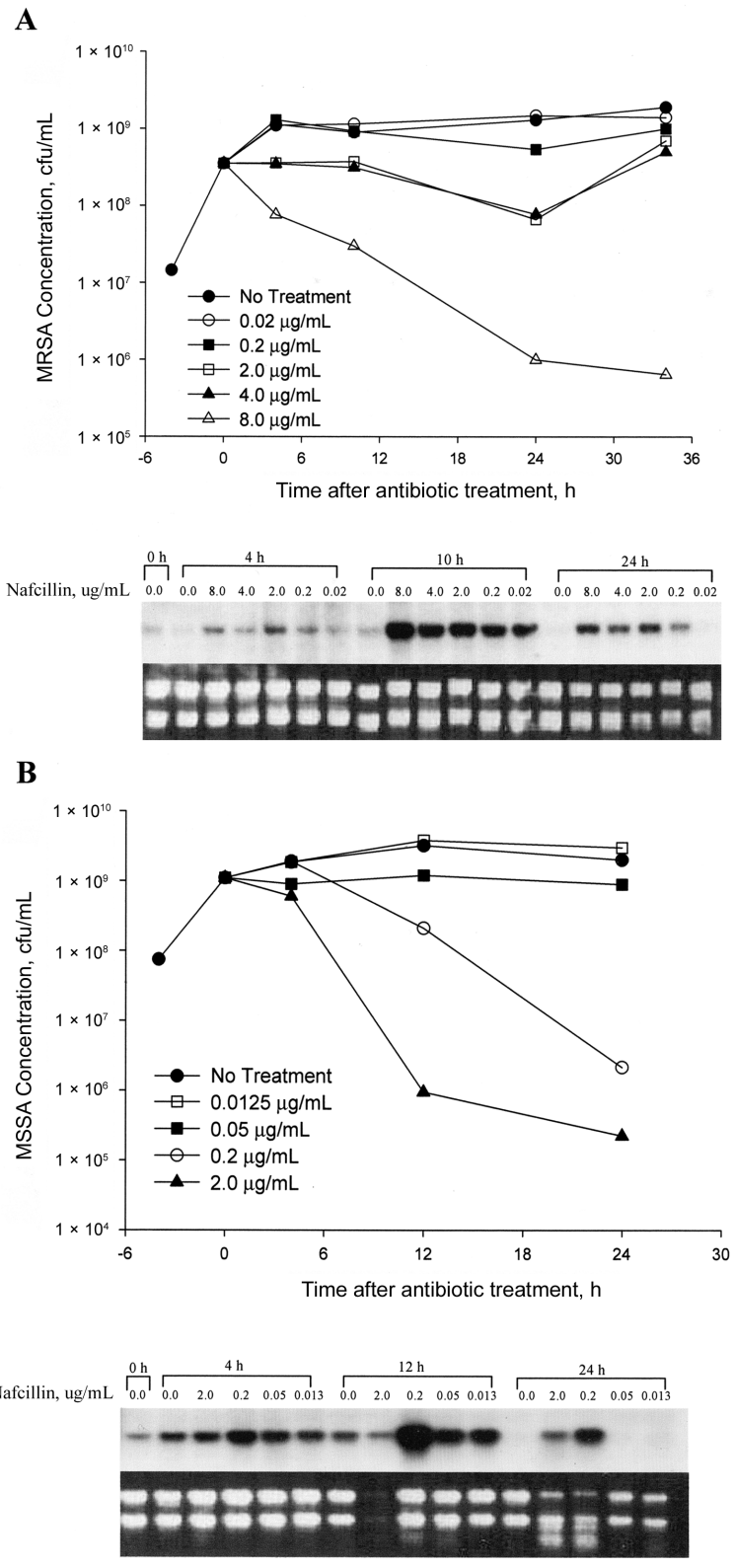

Figure 3. Effects of nafcillin on growth and alpha-hemolysin mRNA expression in methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) strains. Nafcillin-treated cultures were sampled at various times over 34 h, for quantitation of bacterial growth and analysis of alpha-hemolysin gene expression by northern blotting, as described in Materials and Methods. Data shown are from 1 representative experiment of 3 for MRSA strain 04-014 *(A)* and of 4 for MSSA strain ATCC 18032 *(B)*. Data for MRSA and MSSA concentrations represent the means of duplicate plate counts at each time point; the discrepancy between replicates is too small to be visible.

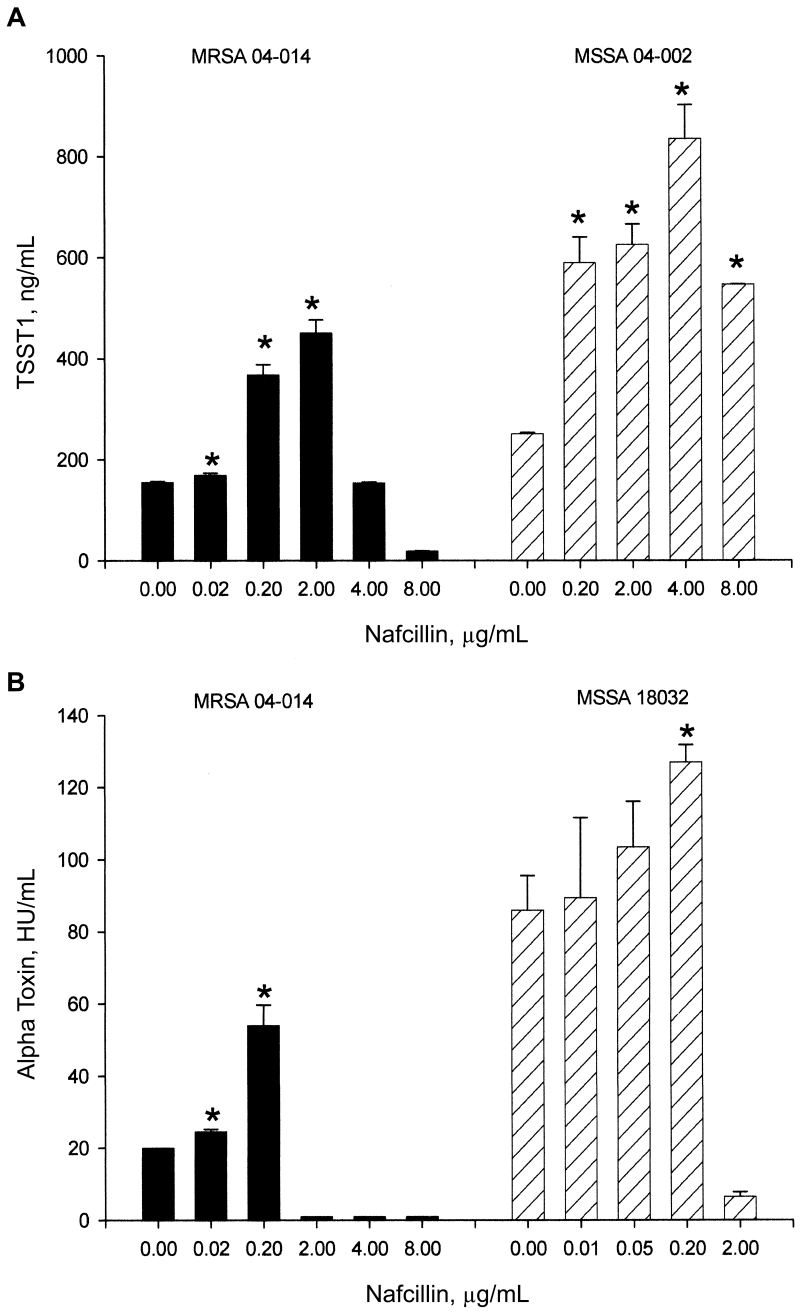

Figure 4. Dose-dependent effects of nafcillin on toxic-shock syndrome toxin 1 (TSST-1) and alpha-hemolysin production in methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA). Nafcillin at the indicated final concentrations was added to mid-log–phase cultures of MRSA and MSSA strains. Production of alpha-hemolysin and of TSST-1 24 h after nafcillin treatment was measured by rabbit red-cell hemolysis assay and ELISA, respectively. Data shown are from 1 representative experiment of 3 or 4 with each strain and are presented as the mean ± SD of duplicate measurements of a single sample per time point. An asterisk (*) denotes that, for the nafcillin concentration indicated, the value for the treated sample was statistically greater (i.e., $P < .05$, by Student's *t* test) than that for the untreated control sample.

41], and we have recently shown that suppression of TSST-1 by clindamycin and linezolid is associated with an excellent clinical response in a patient with STSS due to MSSA [18].

In sharp contrast to the protein-synthesis inhibitors, beta-lactam antibiotics may fail in infections caused by toxin-producing organisms [35–37]. Several reasons for such failure have been elucidated. First is the "Eagle effect," which demonstrates that beta-lactam antibiotics are ineffective during the stationary phase of bacterial growth—or, conversely, that they are only effective in rapidly growing organisms [42]. Eagle hypothesized, and later demonstrated in mice, that such failure was related to the unique "physiologic state of the organism" when large

numbers of organisms were present in the tissue. Specifically, he demonstrated that penicillin remained effective until the organisms reach a point at which they no longer proliferated (i.e., ~10^8 cfu/g tissue). We subsequently showed that this penicillin-refractory state occurs during stationary phase in vitro and is due to down-regulation of PBPs [43]. Extrapolating this in vitro phenomenon to actual infection, one can hypothesize, for GAS infections at least, that organisms may not be rapidly proliferating when a diagnosis of severe infection is made and treatment is initiated. This may be one reason why source control or surgical debridement is profoundly important in deep streptococcal, clostridial, and staphylococcal infections.

The second reason that beta-lactam antibiotics may fail in infections associated with toxin-producing organisms is that these cell-wall–active agents, in contrast to protein-synthesis inhibitors, fail to suppress toxin production. The third reason is that they can increase TSST-1 and alpha-hemolysin [16], owing, in part, to lysis of the organism and release of intracellular toxin. The fourth reason is that subinhibitory concentrations of beta-lactam antibiotics actually increase exotoxin-gene expression [17, 44]. The present study substantiated this finding for alpha-hemolysin and TSST-1 in MSSA and further demonstrated that nafcillin also increased mRNA for alpha-hemolysin, TSST-1, and PVL in MRSA. Not only was PVL mRNA up-regulated by nafcillin, but its expression remained elevated for up to 34 h after antibiotic treatment and was associated with continued toxin production. In contrast, vancomycin had little effect on PVL production compared with that in controls, despite its ability to up-regulate mRNA. These data suggest that inhibition of cell-wall synthesis per se is not the cause of increased toxin production. Although clindamycin and linezolid also prolonged expression of mRNA of alpha-toxin, TSST-1, and PVL, they dramatically curtailed toxin production. Such toxin suppression is consistent with their known mechanism of action—that is, inhibition of protein synthesis at the ribosomal level.

Taken together, these data suggest that, for treatment of staphylococcal infections that are clearly related to production of potent extracellular toxins (e.g., STSS and scalded-skin syndrome), protein-synthesis inhibitors such as linezolid or clindamycin may have beneficial effects on outcomes by attenuating of virulence-factor expression by *S. aureus*. In the treatment of MRSA infections—particularly necrotizing fasciitis and necrotizing pneumonia, which may be attributable to PVL production—linezolid and, potentially, clindamycin (in the absence of inducible resistance) may be useful and of more benefit than is vancomycin. In contrast, inadvertent use of beta-lactam antibiotics to treat TSST-1 or PVL-associated MRSA infections may contribute to worse outcomes by inducing and prolonging toxin production. This could, in part, explain the increased morbidity and mortality associated with MRSA infections compared with MSSA infections as reported by Cosgrove et al (6) since up to 76% of MRSA infections had initially been treated with inappropriate antibiotics (45, 46).

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