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Effects of Subinhibitory Concentrations of Antibiotics on Alpha-Toxin (*hla*) Gene Expression of Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus* Isolates

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Concentrations of antibiotics below the MIC are able to modulate the expression of virulence-associated genes. In this study, the influence of subinhibitory doses of 31 antibiotics on the expression of the gene encoding the staphylococcal alpha-toxin (*hla*), a major virulence factor of *Staphylococcus aureus*, was investigated with a novel gene fusion protocol. The most striking observation was a strong induction of *hla* expression by subinhibitory concentrations of β-lactams and an almost complete inhibition of alpha-toxin expression by clindamycin. Whereas glycopeptide antibiotics had no effect, the macrolide erythromycin and several aminoglycosides reduced and fluoroquinolones slightly stimulated *hla* expression. Furthermore, Northern blot analysis of *hla* mRNA and Western blot (immunoblot) analysis of culture supernatants of both methicillin-sensitive and methicillin-resistant *S. aureus* strains revealed that methicillin-induced alpha-toxin expression is a common phenomenon of alpha-toxin-producing strains. Some methicillin-resistant *S. aureus* isolates produced up to 30-fold more alpha-toxin in the presence of 10 μg of methicillin per ml than in its absence. The results indicate that the novel gene fusion technique is a useful tool for studying the modulation of virulence gene expression by antibiotics. Moreover, the results suggest that the effects of certain antibiotics on virulence properties may be relevant for the management of *S. aureus* infections.

There is increasing evidence that subinhibitory concentrations of antibiotics interfere with processes of host-parasite interactions such as phagocytosis, adherence, and toxin production (24). Several virulence-associated determinants of the important human pathogen *Staphylococcus aureus* are affected in vitro by low levels of various antibiotics (5, 11, 12, 26, 34). Remarkably, subinhibitory concentrations of β-lactam antibiotics, which are the preferred agents in antistaphylococcal chemotherapy, induce the hemolytic activity of *S. aureus* strains, probably via increased alpha-toxin production (12, 18, 23, 39).

The pore-forming alpha-toxin (encoded by the *hla* gene) is a major virulence factor of *S. aureus*. Its role in pathogenesis has been demonstrated in several animal models with Hla-negative mutants (6, 28, 30, 31). Hla exhibits cytolytic, hemolytic, dermonecrotic, and lethal activities (2, 35). In addition, the generation of transmembrane pores triggers calcium-dependent and -independent secondary cellular reactions, such as eicosanoid production, release of cytokines, and apoptosis (3, 9, 36, 37). Many cell types, including erythrocytes, monocytes, lymphocytes, macrophages, epithelial cells, fibroblasts, and keratinocytes, are susceptible to the action of the toxin (2, 4, 40, 41).

Recently, it was shown that growth of *S. aureus* strains in the presence of the β-lactam nafcillin induces alpha-toxin expression and increases the lethal activity of broth filtrates in a murine model (16). These findings led to the speculation that β-lactam therapy may enhance the virulence of some *S. aureus* strains, in turn worsening the symptoms of serious *S. aureus* infections (16). On the other hand, subinhibitory concentrations of antibiotics which interfere with the protein synthesis machinery repressed the hemolytic activity of some *S. aureus* strains (18, 26). However, *S. aureus* produces four hemolysins, and most reports investigating the effect of antibiotics on hemolysis did not clearly show that the modulation of hemolytic activity by antibiotics was due to the action of alpha-toxin.

The aim of this study was a comprehensive characterization of specific effects of subinhibitory concentrations of antibiotics on the expression of the alpha-toxin gene of *S. aureus* by use of a recently described chromosomally located *hla*::*lacZ* gene fusion (29). In addition, the effects of β-lactams upon the transcription and translation of alpha-toxin were examined by Northern hybridization and immunoblotting analysis of both methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) isolates.

MATERIALS AND METHODS

Strains. The bacterial strains used in this study are listed in Table 1. Wood 46-3 is a derivative of Wood 46 (13) carrying a transcriptional fusion between the promoter region of the alpha-toxin gene and the *lacZ* gene of *Escherichia coli* (29). Clinical isolates of *S. aureus* were recovered from patients in Mainz, Frankfurt am Main, and Würzburg, Germany. Each isolate was identified as a unique *S. aureus* strain by established methods (22). The classification of *S. aureus* strains as methicillin sensitive (MIC, ≤8 μg/ml) or methicillin resistant (MIC, ≥16 μg/ml) was carried out in accordance with the criteria of the National Committee for Clinical Laboratory Standards (27). TX71 is a constitutively β-galactosidase-producing *S. xylosus* strain carrying a chromosomal fusion between the *vegII* promoter of *Bacillus subtilis* and the *S. xylosus* β-galactosidase gene *lacH* (7).

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TABLE 1. Bacterial strains used

Strain	Description	Source or reference
S. aureus		
Wood 46	High-level production of alpha-toxin	13
Wood 46-3	Derivative of Wood 46 with *hla*::*lacZ* fusion	29
MA12	MSSA, mucosal isolate	Nursing staff
MA13	MSSA, wound infection	Clinical isolate
MA14	MRSA, wound infection	Clinical isolate
MA15	MRSA, wound infection	Clinical isolate
MA17	MRSA, wound infection	Clinical isolate
MA19	MSSA, wound infection	Clinical isolate
MA20	MRSA, wound infection	Clinical isolate
MA23	MSSA, wound infection	Clinical isolate
MA25	MSSA, wound infection	Clinical isolate
MA31	MRSA, sepsis	Clinical isolate
MA32	MRSA, sepsis	Clinical isolate
W570	MRSA, sepsis	Clinical isolate
W605	MRSA, sepsis	Clinical isolate
W654	MRSA, orthopedic implant-associated infection	Clinical isolate
W655	MRSA, orthopedic implant-associated infection	Clinical isolate
W704	MRSA, pneumonia	Clinical isolate
W724	MRSA, osteomyelitis	Clinical isolate
W810	MRSA, sepsis	Clinical isolate
W903	MRSA, orthopedic implant-associated infection	Clinical isolate
S. xylosus TX71	Constitutive β-galactosidase production	7

Media. For RNA extractions as well as for exoprotein analysis, *S. aureus* strains were cultured in brain heart infusion broth (Difco, Augsburg, Germany). For reporter gene studies, strain Wood 46-3 was cultivated in modified Luria-Bertani (LB) broth consisting of 1% peptone (Roth, Karlsruhe, Germany), 0.5% yeast extract (BRL, Eggenstein, Germany), 0.5% NaCl (Roth), and 0.1% K_2HPO_4 (E. Merck AG, Darmstadt, Germany).

Bacterial growth conditions. *S. aureus* Wood 46-3 was cultivated following a 1:100 dilution of an overnight culture in 100-ml flasks that contained 20 ml of modified B broth. The cultivation was performed with a shaker at 180 rpm and 37°C. As alpha-toxin expression is growth phase dependent, with maximal expression in the late logarithmic to early stationary phases, the cultures were monitored with regard to β-galactosidase activity until the early stationary phase (19, 29). Following the cultivation of clinical isolates under the same conditions, cells were collected for RNA extraction and supernatants were collected for immunoblot analysis.

Antibiotics. The antibiotics used in this study are listed in Table 2.

β-Lactamase tests. The β-lactamase production of the strains was determined by use of β-lactamase identification sticks (Oxoid, Wesel, Germany) with nitrocefin as the substrate.

β-Galactosidase assays. β-Galactosidase assays were performed as described previously (29) with the Galacto Light Plus chemiluminescent reporter assay system (Tropix, Bedford, Mass.). β-Galactosidase activity was measured by use of an LB 9501 luminometer (Berthold, Wildbad, Germany) with a 300-μl automatic injector and a 5-s interval.

Extraction of RNA and DNA-RNA hybridization. RNA was prepared with the RNeasy system (Qiagen, Hillen, Germany). After electrophoresis of samples with the same amount of total cellular RNA, as determined by measuring the A_{260}, the gel was analyzed by Northern blot hybridization as described previously (1, 42). The probe, a 722-nucleotide intragenic *Cla*I fragment (13) from the *hla* gene, was labelled by use of an ECL kit (Amersham, Braunschweig, Germany), and hybridization was performed as described in the manufacturer's instructions. The signals were quantified by densitometric scanning.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed as described by Laemmli (21) with discontinuous 12.5% acrylamide gels. For immunoblot analysis, proteins were transferred to nylon membranes by semidry electroblotting in a graphite chamber (20). Following blotting of the membranes, blocking was performed with 5% nonfat dry milk (Bio-Rad, Munich, Germany) in phosphate-buffered saline for 1 h. The filters were then incubated for 1 h with a polyclonal anti-alpha-toxin antibody in phosphate-buffered saline containing 0.05% Tween 20 (Sigma, Deisenhofer, Germany) followed by 0.5 h of incubation with horseradish peroxidase-conjugated anti-rabbit antiserum (DAKO, Hamburg, Germany) diluted 1:1,000. The blots were developed with ECL substrate (Amersham), and the signals were quantified by densitometric scanning.

Determination of the MICs. The MICs were determined by the broth microdilution method (27), and the results are shown in Table 2.

Statistics. Means ± standard deviations were calculated by the method described by Cavalli-Sforza (8). The values obtained with each antibiotic were compared to those obtained for the control without antibiotic by an unpaired *t* test. *P* values of ≤0.05 were judged significant.

RESULTS

Effects of β-lactams on *hla*::*lacZ* expression. First, the expression of the *hla*::*lacZ* fusion in *S. aureus* Wood 46-3 was examined after growth with subinhibitory concentrations (one-fourth the MIC) of the following penicillin derivatives: ampicillin, azlocillin, cloxacillin, flucloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V, and piperacillin. The highest level of β-galactosidase production, representing *hla* expression, was obtained after growth in the presence of penicillin V, cloxacillin, penicillin G, flucloxacillin, and methicillin (Fig. 1A). The β-galactosidase activity was elevated seven- to eightfold compared to that in a control culture grown without

TABLE 2. MICs of antibiotics for *S. aureus* Wood 46-3

Antibiotic (source)	Abbreviation	MIC (μg/ml)
β-Lactams		
Ampicillin (Sigma)	AMPC	0.125
Azlocillin (Sigma)	AZLC	0.5
Cloxacillin (Fluka, Deisenhofen, Germany)	CLOC	0.25
Flucloxacillin (SmithKline Beecham, Munich, Germany)	FLUC	0.25
Methicillin (Sigma)	METC	1
Nafcillin (Sigma)	NAFC	0.25
Oxacillin (Sigma)	OXAC	0.25
Penicillin G (Sigma)	PENG	0.06
Penicillin V (Sigma)	PENV	0.06
Piperacillin (Fluka)	PIPC	0.5
Cefazolin (Sigma)	CFAZ	0.5
Cefuroxime (Sigma)	CROX	0.5
Cefotaxime (Roussel Uclaf, Romainville, France)	CTAX	0.5
Ceftriaxone (Sigma)	CTRX	1
Cefoxitin (Merck Sharp & Dohme, Haar, Germany)	CFOX	4
Imipenem (MSD) (Merck Sharp & Dohme)	IMIP	0.03
Aztreonam (Bristol-Myers Squibb, Regensburg, Germany)	AZTR	>128
Glycopeptides		
Teicoplanin (Roussel Uclaf)	TPL	1
Vancomycin (Sigma)	VAN	1
Aminoglycosides		
Gentamicin (Sigma)	GEN	4
Kanamycin (Sigma)	KAN	16
Netilmicin (Sigma)	NET	2
Streptomycin (Sigma)	STR	16
Tobramycin (Sigma)	TOB	2
Fluoroquinolones		
Ciprofloxacin (Difco)	CIPX	0.25
Ofloxacin (Sigma)	OFLX	0.5
Macrolide: erythromycin (Sigma)	ERY	1
Other classes		
Clindamycin (Sigma)	CLI	0.25
Rifampin (Sigma)	RIF	0.008
Tetracycline (Sigma)	TET	0.5
Trimethoprim (Sigma)	TMP	0.25

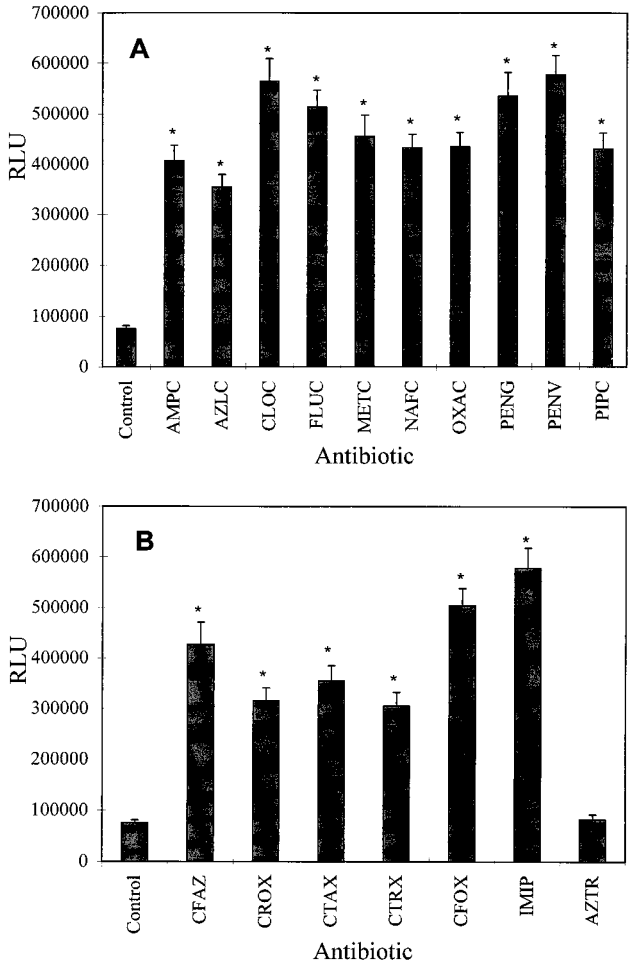

FIG. 1. Influence of subinhibitory concentrations (one-fourth the MIC) of penicillins (A) and other β-lactam antibiotics (B) on β-galactosidase (LacZ) production of the fusion strain *S. aureus* Wood 46-3. Wood 46-3 cultures grown without and with antibiotics were monitored with regard to LacZ production until the early stationary phase. Maximal LacZ values are given as relative light units (RLU). Means ± standard deviations for five experiments are given. The values obtained with each antibiotic were compared to those obtained for the control without antibiotic by an unpaired *t* test: *, $P \leq 0.01$; the other differences were not statistically significant ($P > 0.05$). The abbreviations are listed in Table 2.

any antibiotic. The remaining penicillins induced *hla*::*lacZ* expression five- to sixfold (Fig. 1A).

To determine if β-lactam antibiotics other than penicillins also influence *hla* expression, β-galactosidase activity in strain Wood 46-3 was examined after growth with one-fourth the MIC of cephalosporins (cefazolin, cefuroxime, cefotaxime, ceftriaxone, and cefoxitin), the carbapenem imipenem, and the monobactam aztreonam. As shown in Fig. 1B, all of these antibiotics, except for aztreonam, induced β-galactosidase activity. The greatest extent of induction was obtained after growth with imipenem (eightfold) and cefoxitin (sevenfold). The other tested cephalosporins increased *hla*::*lacZ* expression four- to fivefold. In contrast, aztreonam had no effect on β-galactosidase production (Fig. 1B).

None of the β-lactams tested had any influence upon growth-phase-dependent induction of alpha-toxin expression. Although subinhibitory levels of β-lactams, except for aztreonam, slightly decreased the growth of Wood 46-3, the induction of alpha-toxin expression occurred during the late exponential phase of growth, and the maximal level of alpha-toxin expression was detected in the early stationary phase. A representative growth curve for strain Wood 46-3 grown in the presence of methicillin and the corresponding LacZ values are shown in Fig. 2A.

Effects of glycopeptide antibiotics and aminoglycosides on *hla*::*lacZ* expression. The influence of one-fourth the MIC of two glycopeptide antibiotics (vancomycin and teicoplanin) and five aminoglycosides (gentamicin, kanamycin, netilmicin, streptomycin, and tobramycin) on *hla* expression was investigated. The glycopeptide antibiotics did not significantly ($P > 0.05$) affect *hla*::*lacZ* expression (Fig. 3A). However, all of the aminoglycosides tested decreased *hla*::*lacZ* expression significantly ($P \leq 0.01$): tobramycin, 54%; netilmicin, 46%; streptomycin, 40%; gentamicin, 34%; and kanamycin, 23% (Fig. 3A).

Effects of clindamycin, erythromycin, tetracycline, rifampin, fluoroquinolones, and trimethoprim on *hla*::*lacZ* expression. Low concentrations of clindamycin strongly inhibited β-galactosidase activity (Fig. 3B). This antibiotic reduced *hla*::*lacZ* expression by 98% compared with that in a control culture. The repression occurred during the whole growth cycle (Fig. 2B). Further, erythromycin had a marked repressive effect on *hla* promoter activity. While rifampin and tetracycline slightly

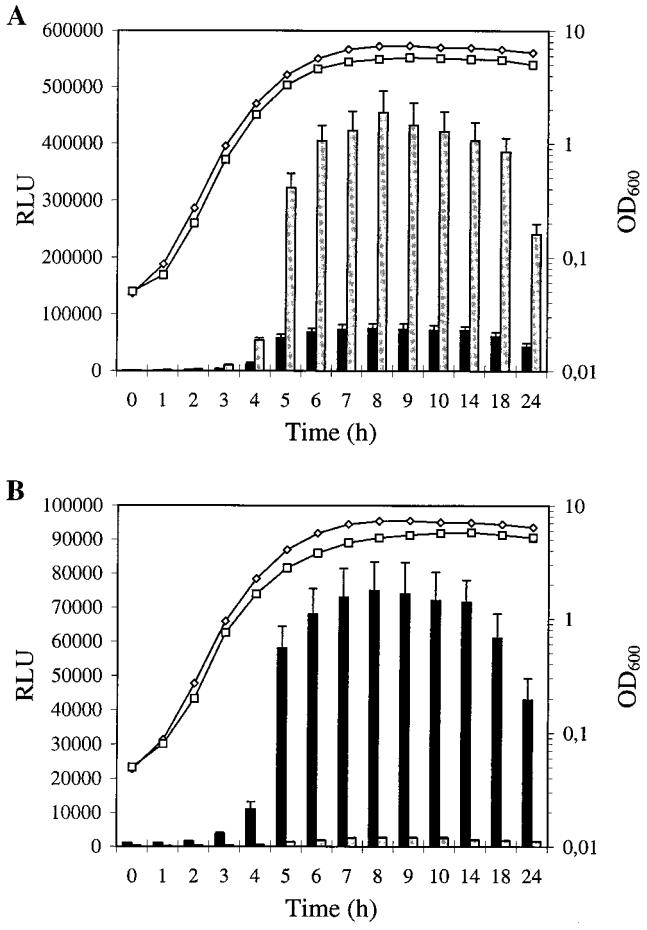

FIG. 2. Kinetics of transcription of a chromosomal *hla*::*lacZ* fusion with growth in the presence of methicillin (A) and clindamycin (B) (drugs are indicated by gray bars and open squares) and a control without antibiotic (black bars and open diamonds). β-Galactosidase activity, indicated by bars, is expressed in relative light units (RLU). Means ± standard deviations for four experiments are given. Lines with squares and diamonds indicate representative growth, as measured by the optical density at 600 nm (OD_{600}). Growth experiments were repeated four times.

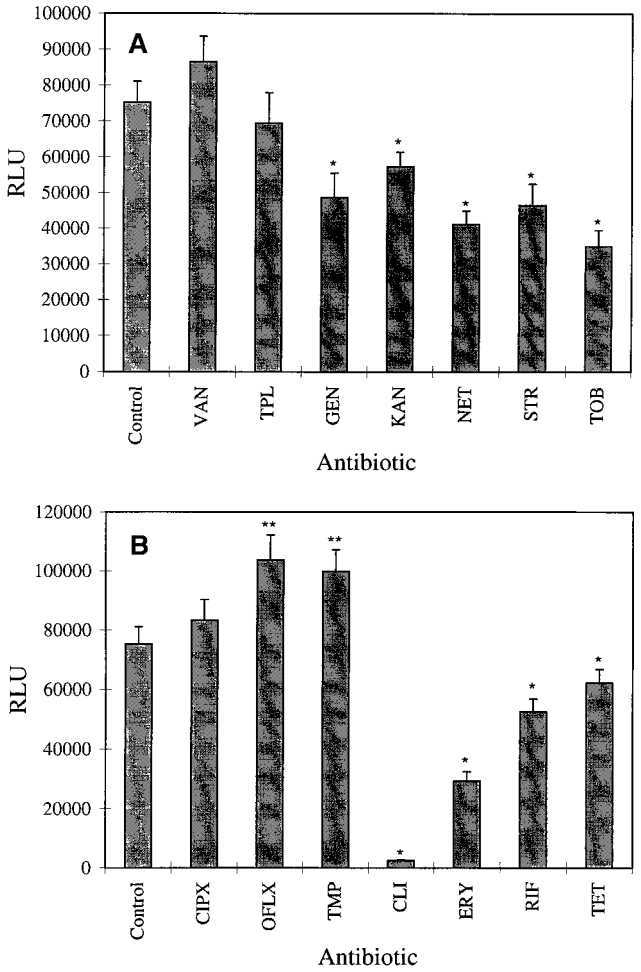

FIG. 3. Influence of subinhibitory concentrations (one-fourth the MIC) of glycopeptides and aminoglycosides (A) and various antibiotics (B) on β-galactosidase (LacZ) production of the fusion strain *S. aureus* Wood 46-3. Wood 46-3 cultures grown without and with antibiotics were monitored with regard to LacZ production until the early stationary phase. Maximal LacZ values are given as relative light units (RLU). Means ± standard deviations for five experiments are given. The values obtained with each antibiotic were compared to those obtained for the control without antibiotic by an unpaired *t* test: *, $P \leq 0.01$; **, $P \leq 0.05$; the other differences were not statistically significant ($P > 0.05$). The abbreviations are listed in Table 2.

decreased the β-galactosidase production of the reporter strain, the fluoroquinolone ofloxacin and trimethoprim slightly increased *hla*::*lacZ* expression ($P \leq 0.05$) (Fig. 3B).

Influence of methicillin on *hla* mRNA expression and alpha-toxin production of MSSA isolates. The effect of β-lactam-induced alpha-toxin expression was further investigated with MSSA strains on both transcriptional and translational levels. First, total RNAs of six strains were analyzed by DNA-RNA hybridization and quantified by densitometric scanning after cultivation with one-fourth the MIC of methicillin (0.25 μg/ml). All strains produced higher *hla* mRNA levels after growth with than after growth without methicillin (Fig. 4). However, the increase was strain specific, ranging from 1.5- to 10-fold.

To determine whether the effect of methicillin on *hla* expression is also associated with an increase in alpha-toxin production, six MSSA strains were cultivated in the absence or presence of methicillin (0.25 μg/ml). The alpha-toxin concentration in supernatants was displayed by immunoblot analysis and quantified by densitometric scanning. Growth in the presence of methicillin elevated the alpha-toxin level of all strains tested (Fig. 5). Methicillin caused an average 4.5-fold increase in alpha-toxin production. However, while strain MA19 produced 12-fold more alpha-toxin after growth with than after growth without methicillin, the increase in strain MA12 was only 1.5-fold, indicating that strain-specific regulatory mechanisms which determine the extent of induction of alpha-toxin production by methicillin exist.

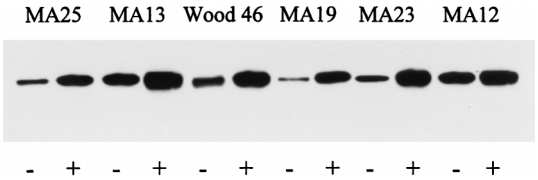

FIG. 4. Northern blot analysis of total RNA from MSSA strains after growth without (−) and with (+) methicillin (0.25 μg/ml). RNA was prepared from cells after 8 h of growth in modified LB broth. Six micrograms of RNA was loaded into each well. After electrophoresis and blotting, the filter was probed for *hla* mRNA with a peroxidase-labelled 722-nt intragenic *hla*-specific *Cla*I fragment.

Influence of methicillin on alpha-toxin production of MRSA isolates. Methicillin increased the alpha-toxin production of MSSA strains. Therefore, we examined the effect of methicillin on methicillin-resistant strains. First, MRSA strain MA17 (MIC, 1 mg/ml) was grown with different concentrations of methicillin (0.1 to 500 μg/ml), and culture supernatants were analyzed by immunoblotting. Methicillin at 0.1 μg/ml elevated alpha-toxin production threefold, and growth in increasing concentrations of methicillin was accompanied by increasing alpha-toxin levels in culture supernatants (Fig. 6). However, the alpha-toxin levels reached a plateau at about 5 to 10 μg of methicillin per ml. Growth in higher methicillin concentrations did not stimulate alpha-toxin production further. Strain MA17 produced about 15-fold more alpha-toxin at maximal induction than the respective control culture grown without methicillin.

The effect of methicillin on the alpha-toxin production of four additional MRSA strains was analyzed by immunoblotting. Since strain MA17 produced maximal alpha-toxin levels at a methicillin concentration of about 10 μg/ml, these four strains were cultivated with 10 and 50 μg of methicillin per ml and, as a control, without the antibiotic added. As shown in Fig. 7, all strains tested produced dramatically more alpha-toxin in the presence of methicillin than in its absence (control cultures). Strain MA14 exhibited detectable alpha-toxin levels only in the presence of methicillin, and strain MA31 showed a 30-fold increase in alpha-toxin production in the presence of methicillin (quantified by densitometric scanning). In addition, nine additional strains were cultivated in 10 μg of methicillin per ml. These strains also produced more alpha-toxin in the presence of methicillin than in its absence (Table 3).

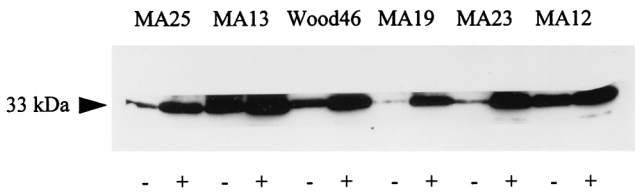

FIG. 5. Immunoblot analysis of alpha-toxin production of MSSA strains after growth without (−) and with (+) methicillin (0.25 μg/ml). Culture supernatant samples were taken following 18 h of incubation, and 10 μl of each sample was loaded onto the gel.

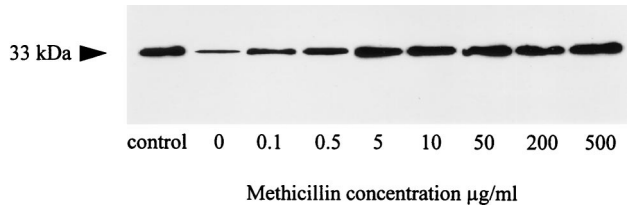

FIG. 6. Immunoblot analysis of alpha-toxin production of MRSA strain MA17 after growth with different concentrations of methicillin. Culture supernatant samples were taken following 18 h of incubation, and 10 μl of each sample was loaded onto the gel. As a control, 1 μg of purified alpha-toxin (Sigma) was used in the control lane.

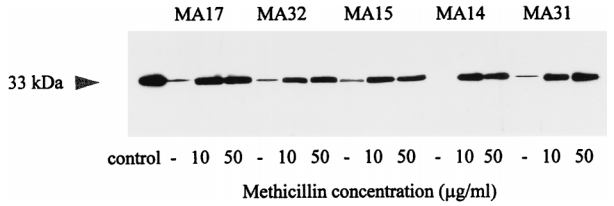

FIG. 7. Immunoblot analysis of alpha-toxin production of MRSA strains after growth without (−) and with methicillin (10 and 50 μg/ml). Culture supernatant samples were taken following 18 h of incubation, and 10 μl of each sample was loaded onto the gel. As a control, 1 μg of purified alpha-toxin (Sigma) was used in the control lane.

DISCUSSION

The present study shows that subinhibitory concentrations of various antibiotics modulate the expression of the *hla* gene, encoding staphylococcal alpha-toxin (Hla). The main findings are that (i) β-lactam antibiotics of different classes strongly induce *hla* expression; (ii) clindamycin almost completely represses *hla* expression; and (iii) methicillin enhances Hla production of both MSSA and MRSA.

Numerous reports have described the effects of antibiotics below the MIC on bacterial cell functions, including alterations of virulence properties (14, 15, 17, 34). In *S. aureus*, exposure to subinhibitory concentrations of antimicrobial agents led to an increased expression of fibronectin-binding proteins by fluoroquinolones (5), an inhibition of toxic shock syndrome toxin 1 (TSST-1) production by clindamycin (32, 38), and an induction of hemolytic activity by β-lactams (12, 18, 23, 39). The last observation frequently has been ascribed to the increased production of alpha-toxin. However, *S. aureus* expresses four hemolysins, and the higher level of hemolytic activity after growth in the presence of β-lactams described in these reports was not entirely due to the action of alpha-toxin.

In this study, we used an *S. aureus* wild-type gene fusion between the *hla* determinant and the reporter gene *lacZ* (29). With this fusion, it was possible to determine specifically the influence of subinhibitory concentrations of various antibiotics of different classes on *hla* promoter activity. It was shown that all penicillins, cephalosporins, and carbapenems tested strongly induced *hla* expression. The highest induction rates were obtained with growth in the presence of penicillin V, penicillin G, cloxacillin, and imipenem, which all have strong activity for susceptible *S. aureus* strains. In contrast, aztreonam, a β-lactam of the monobactam group without antistaphylococcal activity, did not have an effect on *hla* expression. These results suggest that the induction of *hla* expression by β-lactams depends on a specific interaction of the agents with penicillin-binding proteins. As a consequence, such an interaction may induce signal transduction mechanisms, resulting in activation of the *hla* promoter. The details of the induction process, however, remain unknown. Another hypothesis is that there is cross talk between the β-lactamase regulatory system and the virulence regulation machinery in *S. aureus*. However, we found no link between the β-lactamase status of the strains and the induction of alpha-toxin expression by β-lactams. Both β-lactamase-positive strains (e.g., MA17, MA23, MA25, and MA31) and β-lactamase-negative strains (e.g., Wood 46, MA12, MA14, MA15, and MA19) showed increased alpha-toxin production after growth in the presence of subinhibitory concentrations of methicillin.

Further insights into the mechanisms underlying β-lactam-induced *hla* expression were provided by experiments with MRSA strains. Interestingly, methicillin resistance does not prevent the induction process, and methicillin concentrations far below the MIC (10^{-4}) stimulated *hla* promoter activity. Furthermore, the increase in alpha-toxin production with growth in the presence of methicillin was concentration dependent, reaching a maximal level at about 10 μg of methicillin per ml. This finding indicates that the absolute antibiotic concentration rather than the ratio of the concentration to the MIC determines induction. The studies with MRSA strains also indicated that the induction of alpha-toxin expression by β-lactams is a specific process and cannot be explained simply by stress phenomena resulting from destabilization of the bacterial cell wall or the accumulation of cell wall precursors. This hypothesis is further supported by experiments with glycopeptide antibiotics, which interfered with peptidoglycan synthesis but did not alter *hla*::*lacZ* expression. Furthermore, strain-specific regulatory mechanisms determine the extent of the induction. One recent study provided evidence that the activation of *hla* transcription by subinhibitory levels of the β-lactam nafcillin cannot be explained by increased levels of the regulatory molecule RNA III (16). RNA III is the effector of the global regulatory locus *agr* (accessory gene regulator), which controls the expression of a number of virulence genes in *S. aureus*, including alpha-toxin (19). Our observations are consistent with these findings (data not shown).

In contrast, strong inhibition of *hla* expression was observed with growth in the presence of subinhibitory levels of clindamycin. It is noteworthy that low concentrations of clindamycin also inhibit the expression of TSST-1 (32, 38) and the exfoliative toxin (33). With respect to the beneficial effects of clindamycin on TSST-1 production, it has been recommended that clindamycin rather than β-lactams be used for the treatment of staphylococcal toxic shock syndrome (32, 38). Moreover, sublethal concentrations of clindamycin suppress the adhesion

TABLE 3. Alpha-toxin produced by clinical MRSA strains after growth with or without methicillin

Strain	Ratio[a]
MA20	6.2
W570	21.5
W605	12.0
W654	4.4
W655	4.0
W704	18.4
W724	2.5
W810	9.1
W903	2.4

[a] The strains were cultivated either with 10 μg of methicillin per ml or without methicillin. Alpha-toxin levels were determined by immunoblotting and were quantified by densitometry. The ratio shows alpha-toxin produced in the presence of methicillin to that produced in the absence of methicillin.

of *S. aureus* to bone surfaces of rabbits (25) and of *S. epidermidis* to vascular catheters (17) by as-yet-unknown mechanisms. Other protein synthesis inhibitors (erythromycin and aminoglycosides) tested also impaired alpha-toxin expression. However, inhibition by these agents was not as strong as that by clindamycin. Most of the antibiotics tested also slightly reduced the growth of the indicator strain, Wood 46-3, and it may be hypothesized that protein synthesis inhibitors in particular may influence toxin expression by slowing the growth rate. Growth effects may be somewhat involved in the decrease in *hla* expression by aminoglycosides. However, the strong inhibition of *hla* expression by clindamycin cannot be explained simply by a decrease in the growth rate, since very low clindamycin concentrations (below one-fourth the MIC), which did not influence growth, led to a strong inhibition of alpha-toxin expression (data not shown). In addition, clindamycin did not influence the β-galactosidase expression of constitutively β-galactosidase-producing *S. xylosus* TX71, whereas aminoglycosides slightly reduced β-galactosidase production in this strain (data not shown). Thus, clindamycin seems to affect toxin biosynthesis selectively without shutting off ribosomal protein biosynthesis completely.

The contribution of antibiotic-based alteration of alpha-toxin production to the pathogenesis of serious infections caused by *S. aureus* is difficult to evaluate. In the management of *S. aureus* infections, β-lactam antibiotics are the preferred class of drugs. However, we have shown that clinically achieved concentrations of β-lactams induce the production of a major staphylococcal virulence factor. Thus, *S. aureus* strains which do not respond to β-lactam therapy may show enhanced virulence potential, which in turn may lead to an unfavorable impact on the outcome of an infection. Further, the data support the value of high-dose regimens for the treatment of infections caused by MSSA strains to avoid a reduction of therapeutic levels of β-lactams below the MICs.

Since most staphylococcal diseases are multifactorial, involving the production of various virulence determinants, further studies, including animal models and clinical trials, are needed to elucidate the effects of antibiotics on the pathogenesis of serious *S. aureus* infections. Moreover, increasing problems with multiple-drug-resistant *S. aureus* strains demand a renewed effort to develop effective strategies against this pathogen. In addition to classic antimicrobial chemotherapy, a new approach could be the search for agents which suppress gene products associated with infection (10). With respect to this challenge, sensitive reporter gene-based techniques, such as that described in this report, are valuable screening tools and may help researchers find new effective compounds against molecular targets in *S. aureus*.

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