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Antivirulence Potential of TR-700 and Clindamycin on Clinical Isolates of *Staphylococcus aureus* Producing Phenol-Soluble Modulins[▽]

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Staphylococcus aureus **strains (*n* = 50) causing complicated skin and skin structure infections produced various levels of phenol-soluble modulin alpha-type (PSMα) peptides; some produced more than twice that produced by the control strain (LAC USA300). TR-700 (oxazolidinone) and clindamycin strongly inhibited PSM production at one-half the MIC but exhibited weak to modest induction at one-fourth and one-eighth the MICs, primarily in low producers. Adequate dosing of these agents is emphasized to minimize the potential for paradoxical induction of virulence.**

Secreted exotoxins such as toxic shock syndrome toxin 1 (TSST-1), α-hemolysin (Hla), and Panton-Valentine leukocidin (PVL) have been shown to contribute in part to the virulence of *Staphylococcus aureus* (1, 2, 14). The phenol-soluble modulin alpha-type (PSMα) peptides (1–4) are one of the most recently discovered peptides that have been implicated in the pathogenesis of community-associated methicillin-resistant *S. aureus* (CA-MRSA) complicated skin and skin structure infections (cSSSIs), bacteremia, and pneumonia (2, 16). Like the PVL toxin, the PSM peptides target primarily neutrophils, leading to pore formation and inflammatory mediator release. PSMα3 is the most cytolytic, with 3 to 10 μg/ml shown to cause 25 to 60% lysis of human neutrophils (5). PSMα peptides are secreted as both nonformylated and formylated forms, with the latter at a significantly higher quantity and greater cytotoxicity (16). Previous studies have shown that protein synthesis inhibitors at sub-MICs inhibit the production of Hla, PVL, TSST-1, and other virulence factors in the laboratory and a few clinical *S. aureus* strains (3, 4, 8, 11). We sought to investigate the effects of subinhibitory concentrations of the protein synthesis-inhibiting antibiotics, clindamycin and a second-generation oxazolidinone, TR-700, on PSM production.

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We examined baseline production of PSMα1 to -4 in 50 PVL-positive methicillin-susceptible *S. aureus* (MSSA) and MRSA clinical isolates causing cSSSIs. Quantitation of formyl-PSMα peptides after 24 h of incubation was performed in duplicate by liquid chromatography-tandem mass spectrometry (LC-MS-MS). To assess the effect of antibiotics on PSM production in selected clinical isolates and the LAC (USA300) control strain, we used a modified CLSI broth macrodilution to determine the MIC, in which tryptic soy broth was used as the media and samples were shaken at 250 rpm for 24 h at 37°C. $MIC_{50/90}$ values for TR-700 and clindamycin were 0.25/0.375 and 0.125/0.188 μg/ml, respectively. Supernatants were harvested after incubation with study drugs at one-half, one-fourth, and one-eighth the MICs for PSM quantitation and global regulator (*agrA* and RNAIII) expression analysis by reverse transcription-PCR (RT-PCR) (normalized to expression of *gyrB*).

Baseline PSMα production among 50 clinical isolates varied from 0.22 to 98.24 μg/ml (Fig. 1). Some (14%) isolates produced more than twice the amount of PSMα1 to -4 peptides produced by the LAC strain. PSMα production did not differ by methicillin resistance, type of cSSSIs (cellulitis with or without abscess), or size of the abscess (>5 or ≤5 cm) caused by these strains.

Clinical strains were grouped by baseline PSM production to select for representative high, medium, and low producers for studying the effect of subinhibitory TR-700 and clindamycin on PSM production. Experiments on growth kinetics with or without drugs were performed on the LAC strain and two clinical strains (MRSA and MSSA) (Fig. 2). At one-half the MICs of both drugs, growth was delayed, with a lower final cell count in LAC but to a lesser degree in the clinical isolates selected, whereas minimal to no effect on growth was observed at one-fourth and one-eighth the MICs for both agents. Measured PSMα concentrations were normalized to the number of CFU at the time of harvest to account for differences in growth and cell counts.

Overall, 21 clinical isolates and the LAC strain were tested. TR-700 at one-half the MIC had a pronounced inhibitory effect on PSM production in a dose-dependent manner, though the effect varied for all four alpha subtypes (Fig. 3a). PSMα3 was the most inhibited, in which nearly all isolates tested produced no measurable amounts, while PSMα4 was the least inhibited, with production decreasing to a median of 21% of the baseline value. Interestingly, paradoxical induction of PSMα was observed for TR-700 at one-fourth and one-eighth the MICs affecting primarily strains with low baseline production of PSMα3. The highest level of PSMα3 induced with TR-700 was 4.6 μg/ml from 2.5 μg/ml. Similarly, in the LAC control strain, another low baseline producer of PSMα3, TR-700 at one-half the MIC significantly inhibited PSM produc-

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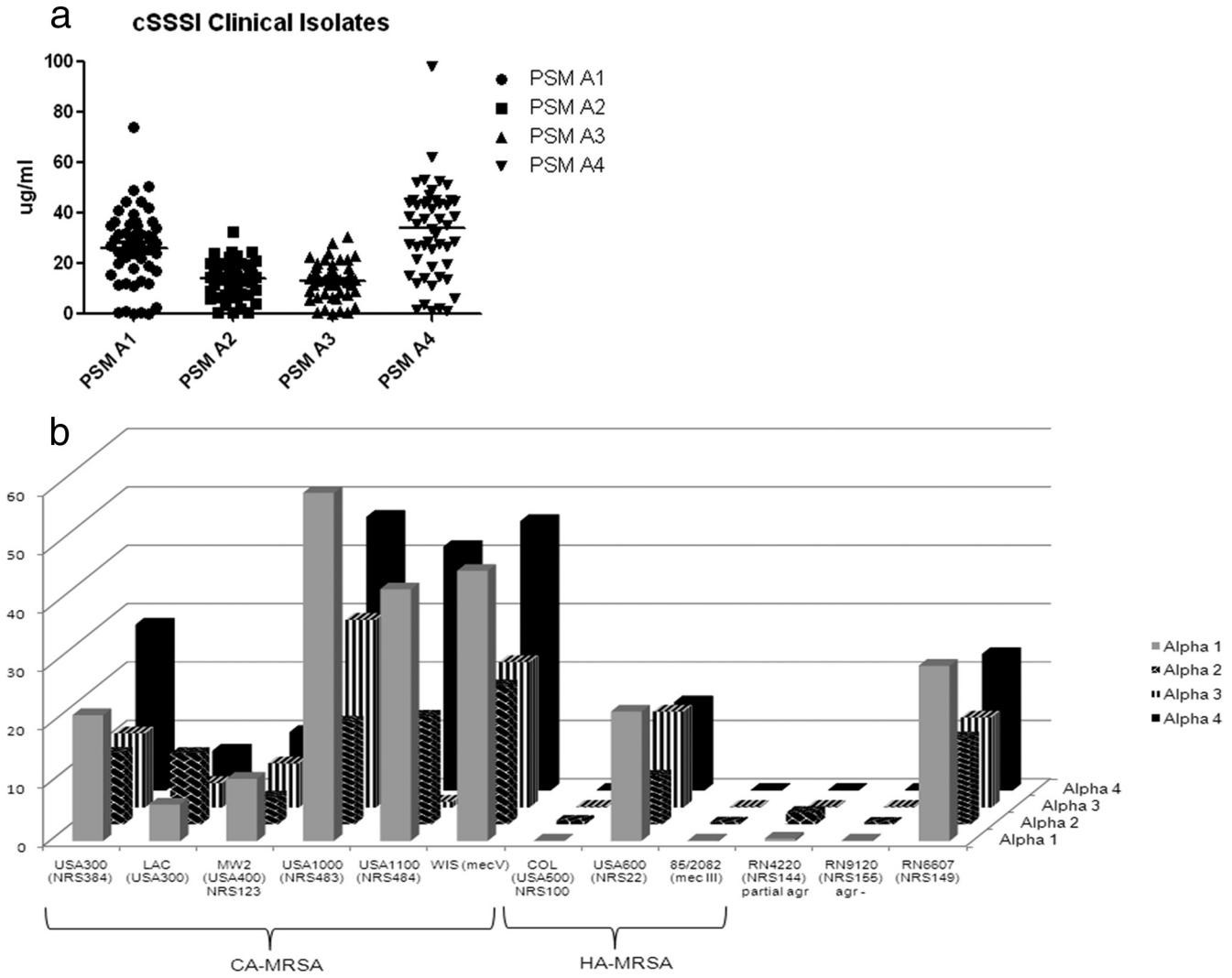

FIG. 1. (a and b) Baseline PSMα1 to -4 production in clinical isolates and reference strains. (a) Clinical isolates that caused cSSSIs (n = 50). Horizontal lines represent median values for each PSMα peptide. The interquartile ranges (IQRs) for PSMα1 to -4 were 16.6 to 35.3, 7.8 to 19.4, 8.5 to 17.5, and 17.5 to 44.6 μg/ml, respectively. (b) *Staphylococcus aureus* control strains. Baseline PSM production of CA, hospital-associated (HA), and laboratory strains of *S. aureus* was measured after 24 h of incubation at 37 °C and shaking at 200 rpm. Laboratory strain NRS155 is the isogenic *agr* knockout of NRS149, and NRS144 has a partial *agr* defect.

tion to 30% of the baseline, while one-fourth and one-eighth the MICs increased PSM production by 40% and 45%, from 3.9 μg/ml to 5.43 and 5.63 μg/ml, respectively.

Compared to TR-700, clindamycin had a stronger inhibitory effect on PSM production overall in a subset of the above-mentioned isolates (n = 7) (Fig. 3b). Complete inhibition of PSMα1 to -4 was observed at one-half the MIC in all but two strains. Against PSMα3, one-fourth the MIC completely inhibited production in 5 of 7 clinical isolates. Like TR-700, PSM production was weakly induced above the baseline level at one-fourth and one-eighth the MICs in two clinical isolates and the LAC strain. Others have documented in the LAC strain that both clindamycin (at 67% of the MIC) and linezolid (at 10% of the MIC) stimulated PSM production by 3.5 and 1.5 times above the baseline, respectively (6).

Like PVL and Hla, PSMs are under strict control of *agrA* and RNAIII of the *agr* system (9, 10, 16). Others have examined the effect of antibiotics on RNAIII expression after 8 h during postexponential growth phase when the *agr* system would be maximally expressed (6). We extended the previous investigation to determine whether the effects persist after overnight incubation. Expression of *agrA* and RNAIII appears to follow the direction of PSM inhibition at sub-MICs of TR-700, even after 24 h (Fig. 4). While clindamycin inhibited both *agrA* and RNAIII, inhibition was less at one-half the MIC than one-eighth the MIC, suggesting differential response of these regulators, or perhaps the inhibitory effect of clindamycin on *agrA* is less sustained over time than that of TR-700.

Our study had limitations. First, we chose subinhibitory concentrations of antibiotics that would likely be encountered in the clinical setting due to improper dosing or other parameters impacting drug levels at the site of infection. However, growth was delayed in some strains at one-half the MIC. We normalized measured PSMα levels to the number of CFU to account

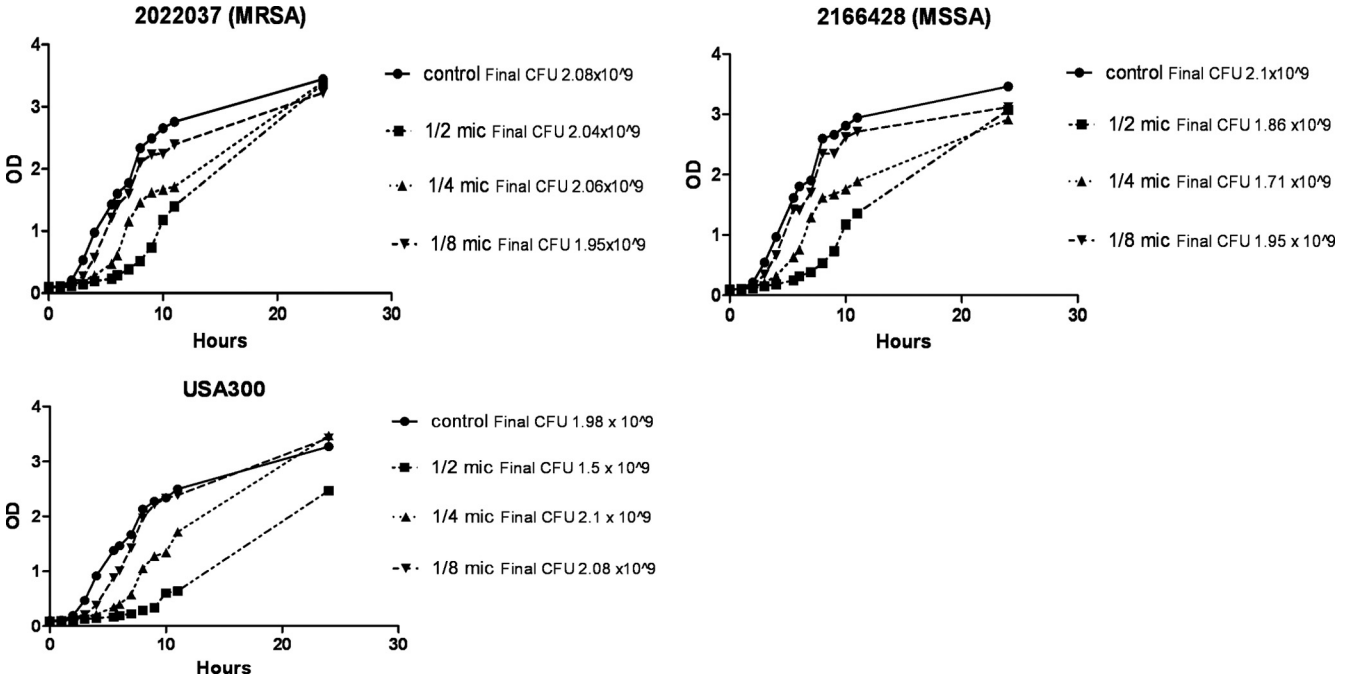

FIG. 2. Growth curves of two clinical isolates (MRSA, MSSA) and the LAC (USA300) control strain at sub-MICs of TR-700. Growth curves with clindamycin were similar (data not shown). Optical densities (ODs) were read every hour for 10 hours, and a final OD reading was taken at 24 hours.

for possible cell count differences, recognizing that this may not completely account for alteration in growth patterns and, in turn, PSM production. Second, we did not obtain samples at earlier time points during growth, which could better characterize responses of global regulators. Finally, the *in vitro* effects of subinhibitory TR-700 and clindamycin will need *in vivo* confirmation to clarify their clinical relevance (13).

Taken together, our findings indicated that *S. aureus* strains causing cSSSIs produced various amounts of PSMα1 to -4. Our study results support the antivirulence potential of protein

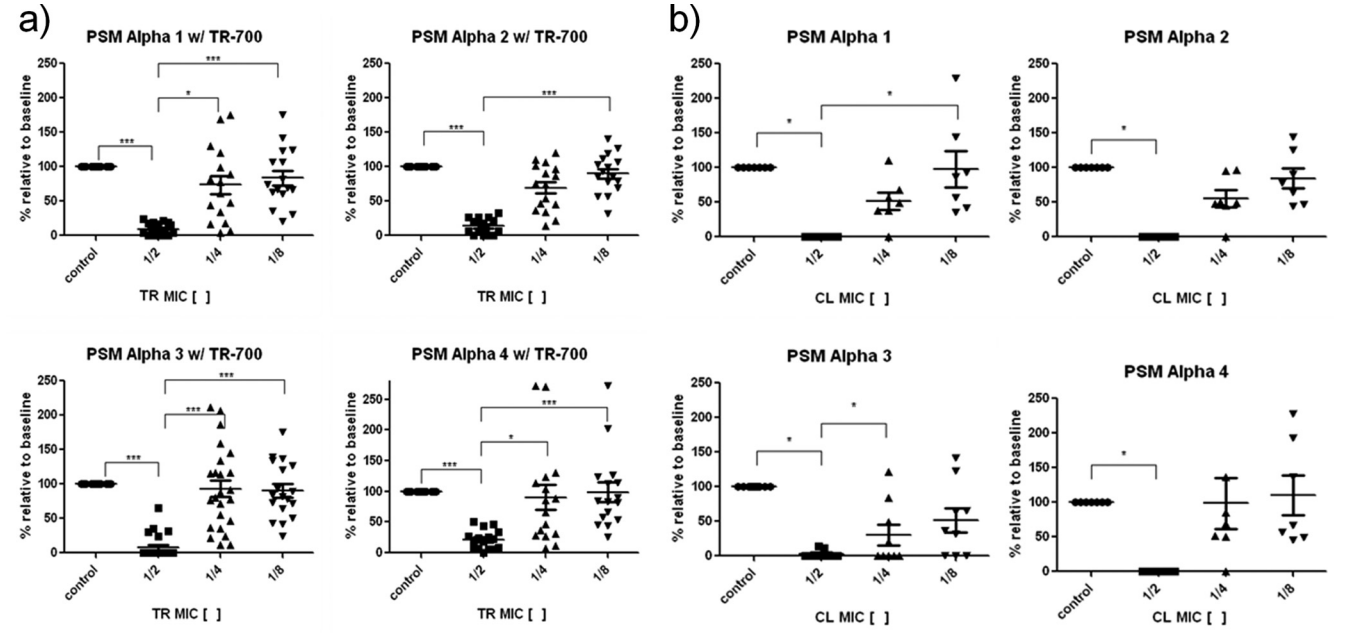

FIG. 3. (a and b) Effect of subinhibitory concentrations of TR-700 (TR) (a) and clindamycin (CL) (b) on PSM production. Data represent the medians with IQRs. One-way analysis of variance (ANOVA) with Dunnett's posttest was used for statistical analysis. Note that an increase in PSM production from the baseline at one-fourth and one-eighth the MICs of TR-700 and clindamycin occurred primarily in low PSM producers. ***, $P < 0.0001$; *, $P < 0.007$.

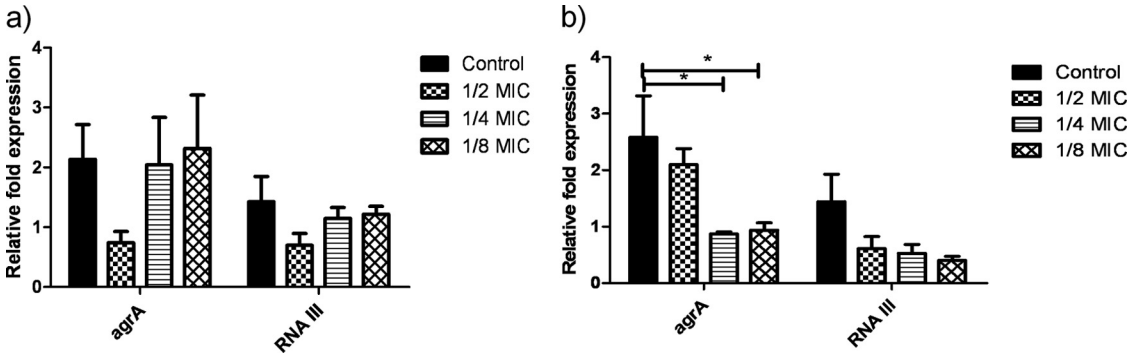

FIG. 4. (a and b) Effect of subinhibitory concentrations of TR-700 (a) and clindamycin (b) on expression of *agrA* and RNAIII. $n = 5$ isolates. Expression is normalized to that of *gyrB*. $*$, $P = 0.017$.

synthesis-inhibiting antibiotics in decreasing PSM production, especially at one-half the MIC, consistent with the published literature on other exotoxins (3, 4, 12, 15). However, it is notable that these antibiotics can cause paradoxical effects on PSM production, albeit a weak to moderate induction predominantly in low PSM producers. Our results underscore the importance of adequate dosing of these agents in order to minimize the potential for paradoxical induction of virulence.

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