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Letter to the Editor

The *Clostridium difficile* quorum-sensing molecule alters the *Staphylococcus aureus* toxin expression profile

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Sir,

Treatment of *Staphylococcus aureus* infections has become increasingly challenging due to the rise of antibiotic-resistant strains. Therefore, development of antibiotic-independent treatments that supplement or provide an alternative approach to traditional therapies is greatly needed. One promising approach is the development of 'antipathogenic' therapies that inhibit bacterial virulence. Quorum sensing is a bacterial communication mechanism in which increasing cell densities precipitate changes in gene expression, allowing bacteria to regulate essential processes [1]. The accessory gene regulator (Agr) quorum-sensing system is a Gram-positive-specific mechanism that is conserved among all staphylococcal species and is similar across a large number of bacteria [2]. It is mediated by small, secreted autoinducing peptides (AIPs) that are usually produced constitutively during growth, and increasing bacterial densities are accompanied by a concomitant increase in AIP concentrations that regulate gene expression [1].

Staphylococcus aureus strains possess any one of four variations of AIPs (groups I to IV) which affect genes that regulate a variety of functions [3], including genes encoding potent pore-forming toxins, immunoevasive compounds, superantigens, and tissue-degrading enzymes associated with severe clinical outcomes [1]. Thus, inhibiting the quorum-sensing mechanism may improve clinical outcomes.

Recently, a *Clostridium difficile* Agr-like quorum-sensing peptide (TI signal) was shown to regulate toxin production [4]. Owing to its similarity with the *S. aureus* AIPs, we examined the effect of the TI signal on *S. aureus* global gene expression using RNA sequencing (RNA-seq). *S. aureus* USA300 (TCH1516, a community-acquired methicillin-resistant strain belonging to Agr group I) was cultured in tryptic soy broth containing 0 (control), 2.85 mg or 22.80 mg of the TI signal for 24 h at 37 °C. Differential gene expression was determined by RNA-seq (SeqWright Genomic Services, Houston, TX.) Total RNA was isolated using RNeasy (QIAGEN, Hilden, Germany) and was quantified on a Nanodrop ND-1000 (Nanodrop, Wilmington, DE). RNA samples were subjected to two rounds of prokaryotic Ribo-Zero rRNA depletion (Illumina, San Diego, CA) and their integrity was evaluated using an Agilent Bioanalyzer RNA Pico chip (Agilent, Santa Clara, CA). cDNA was synthesised using a TruSeq Sample Preparation Kit (Illumina) and was sequenced on the Illumina HiSeq 2000 platform (2×100 bp). Sequencing and statistical analyses were performed using DNAnexus software (DNAnexus, Inc., Mountain View, CA). Data were expressed as fold-change in gene expression compared with the untreated control group (Supplementary Table S1). Fold-changes were statistically significant ($P < 0.0005$) for 28 of the 500 genes for at least one of the TI signal concentrations tested (Supplementary Table S1). Furthermore, the protein expression profiles of strains of different Agr types treated with TI signal were similar, suggesting that the TI signal alters *S. aureus* protein expression independent of the AIP type (Supplementary Fig. S1).

Because the staphylococcal α-toxin gene (*hla*) was significantly affected by the TI signal (Supplementary Table S1), we next examined the impact of the TI signal on α-toxin (strain NRS178) and the Panton–Valentine LukS subunit (LukS-PV) production (strain TCH1516). Bacterial cultures were prepared as above in the presence (0.68 ng to 1.39 μg) or absence of the TI signal for either 24 h or 48 h at 37 °C, respectively. Supernatants were collected and were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) using 4–20% Tris–glycine pre-cast gradient gels (Bio-Rad, Hercules, CA) and were transferred onto nitrocellulose paper. Recombinant LukS-PV [5] and Hla (IBT Bioservices, Gaithersburg, MD) were used as positive controls. Super Block (Pierce, Rockford, IL) was used to block non-specific binding and to dilute the primary and secondary antibodies. Blots were incubated with each antibody for 1 h at room temperature. Blots were washed with 0.05% TBS-T (Tris-buffered saline with 0.05% Tween 20) three times for 5 min each between incubations. The membranes were probed using either rabbit anti-LukS-PV or anti-Hla (IBT Bioservices) at a 1:2000 dilution, followed by goat anti-rabbit alkaline phosphatase-labelled secondary antibody (1:7000 dilution) (Invitrogen, Frederick, MD) [5]. Bands corresponding to respective toxins were visualised following development of the blots with nitro blue tetrazolium chloride/5-bromo-4-chloro-3′-indoly phosphate *p*-toluidine salt (NBT/BCIP) (Pierce). The results demonstrated that the TI signal decreased LukS and Hla production levels in a dose-dependent manner (Fig. 1A). The TI signal also affected the expression profile of staphylococcal protein A (SpA) (Fig. 1A). The observed changes in Hla and LukS production were not due to growth impairment since the doses of the TI signal associated with the lowest CFUs did not correspond to the doses associated with reduced LukS-PV and Hla production (Fig. 1B). Furthermore, the TI signal had no discernible effect on biofilm formation (data not shown).

The present study demonstrated that the *C. difficile* quorum-sensing peptide affected the gene and protein expression profiles of different *S. aureus* strains. Moreover, the TI signal inhibited the production of Hla and LukS-PV toxins important in *S. aureus* pathogenesis, suggesting that the TI signal may be promising as an 'antipathogenic' therapy for *S. aureus* infections. This investigation is ongoing to elucidate the mechanism and to further examine how we could leverage the potential of the TI signal to combat *S. aureus* infections.

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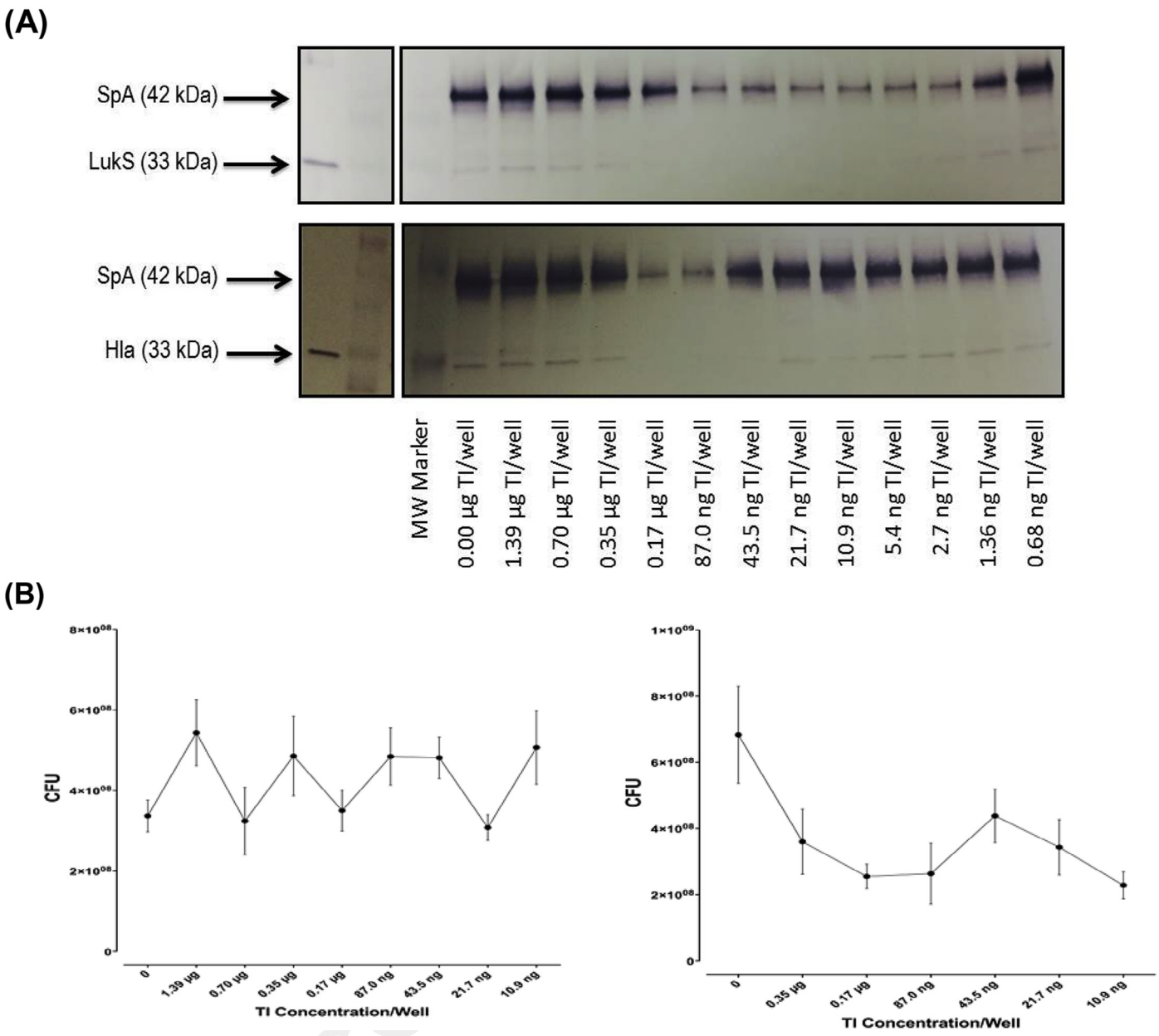

Fig. 1. Effect of the TI signal from *Clostridium difficile* on toxin production and growth of *Staphylococcus aureus*. (A) The TI signal affected the Panton–Valentine LukS subunit (LukS-PV) and staphylococcal α-toxin (Hla) expression profiles of *S. aureus* strains TCH1516 (upper panel) and NRS178 (lower panel). Respective strains were cultured in the absence or presence of decreasing concentrations of TI for 24 h and 48 h, respectively. Supernatants were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) and were subsequently transferred onto nitrocellulose and were probed with either rabbit anti-LukS-PV or anti-Hla, followed by a goat anti-rabbit alkaline phosphatase-conjugated antibody. Blots probed with secondary antibody alone did not show any detectable banding patterns other than non-specific binding to staphylococcal protein A (SpA). Each experiment was repeated twice and representative blots are shown. (B) *S. aureus* growth in the presence of the TI signal. *S. aureus* strains (a) TCH1516 and (b) NRS178 were cultured in the absence or presence of decreasing concentrations of TI signal for 24 h and 48 h, respectively. Pellets were collected and re-suspended in sterile phosphate-buffered saline (PBS), were diluted 1:10^7 and then 100 µL was plated in triplicate onto mannitol salt agar and cultured aerobically at 37 °C. Colonies were counted 24 h later. Data are expressed as the mean CFU per time point tested of three separate experiments for all TI concentrations shown. Graphs were generated and statistical analyses were conducted using GraphPad Prism (GraphPad Software Inc., La Jolla, CA). Student's *t*-test was used to assess statistical differences between CFUs obtained following culture in the presence or absence of the TI signal. No statistical differences between CFUs were observed.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2017.01.001.

References

[1] Yarwood JM, Schlievert PM. Quorum sensing in *Staphylococcus* infections. J Clin Invest 2003;112:1620–5.

[2] Thoendel M, Kavanaugh JS, Flack CE, Horswill AR. Peptide signaling in the staphylococci. Chem Rev 2011;111:117–51.

[3] Vandenesch F, Kornblum J, Novick RP. A temporal signal, independent of *agr*, is required for *hla* but not *spa* transcription in *Staphylococcus aureus*. J Bacteriol 1991;173:6313–20.

[4] Darkoh C, DuPont HL, Norris SJ, Kaplan HB. Toxin synthesis by *Clostridium difficile* is regulated through quorum signaling. MBio 2015;6:e02569.

[5] Brown EL, Bowden MG, Bryson RS, Hulten KG, Bordt AS, Forbes A, et al. Pediatric antibody response to community-acquired *Staphylococcus aureus* infection is directed to the Panton–Valentine leukocidin. Clin Vaccine Immunol 2009;16:139–41.

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