JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Subinhibitory concentrations of tedizolid induce upregulation of virulence gene transcription in *Staphylococcus aureus*

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Abstract

Introduction: Sub-minimal inhibitory concentrations (sub-MICs) of antibiotics can modulate the expression of virulence factors in bacterial pathogens. This study aimed to assess the impacts of sub-MICs of tedizolid on virulence gene expression in *Staphylococcus aureus* (*S. aureus*) and compare them with those of linezolid.

Methodology: Two *S. aureus* strains (N315 and Newman) possessing the selected virulence genes were analyzed. The MICs of tedizolid and linezolid were determined, and sub-MICs for subsequent experiments were selected based on bacterial growth kinetics. Using qRT-PCR, we assessed the expression of 23 virulence genes, including 7 cell wall-anchored (CWA) protein genes, 4 exoenzyme genes, 6 toxin genes, and 6 regulatory genes, before and after exposure to tedizolid and linezolid.

Results: Growth kinetics indicated that 1/8 and 1/4 MICs were optimal for evaluating the influence of drugs on gene expression. The qRT-PCR results revealed that sub-MICs of tedizolid and linezolid primarily enhanced the expression of the studied virulence genes in both strains. In Newman, tedizolid upregulated the expression of more genes encoding CWA proteins, regulators, and toxins than linezolid. In N315, tedizolid stimulated the expression of more toxin-coding genes but fewer regulatory genes compared to linezolid.

Conclusions: Sub-MIC of tedizolid and linezolid could increase the mRNA levels of different types of virulence genes in *S. aureus*, with strain-dependent variations. These findings provide new insights into the potential role of oxazolidinones in bacterial virulence regulation.

Key words: *Staphylococcus aureus*; subinhibitory concentration; tedizolid; linezolid; virulence gene; qRT-PCR.

J Infect Dev Ctries 2025; 19(12):1838-1846. doi:10.3855/jidc.20386

(Received 23 May 2024 – Accepted 16 June 2025)

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Introduction

Staphylococcus aureus (*S. aureus*) is a major human pathogen responsible for a wide range of infectious diseases [1]. In order to establish infections and persist within the host, *S. aureus* produces an array of virulence factors, including surface proteins, extracellular enzymes, toxins, and regulatory molecules controlling pathogenicity [1]. Early investigations demonstrated that the production of these virulence-associated factors can be regulated by subinhibitory doses of antibiotics, which potentially exacerbates clinical outcomes [2,3]. During antibiotic treatment, bacteria face challenges from the drug, and are usually exposed to subinhibitory levels due to factors such as the resistance of the organism, the pharmacokinetics of the antimicrobial agent, and so on [2].

Tedizolid, approved by the FDA in 2014, is a novel (the second-generation) oxazolidinone recommended for the treatment of acute bacterial skin and skin structure infections (ABSSSIs) caused by methicillin-resistant *S. aureus* (MRSA). Compared to linezolid, the first-generation oxazolidinone, tedizolid exhibits more favorable pharmacokinetics and a reduced side effect profile [4,5]. Previous studies have reported that tedizolid significantly reduces the expression of key *S. aureus* virulence factors, including alpha-haemolysin (Hla), Panton-Valentine leukocidin (PVL), phenol-soluble modulin (PSM), and toxic-shock syndrome toxin-1 (TSST-1), both *in vitro* and *in vivo* [6-8]. However, data on tedizolid's broader impact on *S. aureus* virulence gene expression remain limited, as only a few toxins have been investigated [7]. To address this gap, in the current study, we evaluated the *in vitro* effects of subinhibitory concentration of tedizolid on the transcription levels of more selected virulence genes in *S. aureus*, including those encoding surface proteins, toxins, exoenzymes and regulatory factors, using quantitative real-time amplification (qRT-PCR), and

compared with those of subinhibitory levels of linezolid.

Methodology

Bacterial strains

S. aureus strains Newman (methicillin-sensitive strain) and N315 (MRSA), provided by the Institute of Antibiotics, Huashan Hospital, were utilized for experiments in this study. Both strains, stored at -80 °C, were initially streaked onto 5% sheep blood agar plate (Kemajia Microbe Technology Co., Ltd., Shanghai, China) and then cultured in brain heart infusion (BHI) medium (Oxoid, England, UK) for growth and subsequent experiments.

Table 1. Primers used for quantitative real-time polymerase chain reaction (qRT-PCR).

Gene	Encoding protein	Sequence (5'-3')	Reference
Adhesion gene			
clfA-F	Clumping factor A	TGCTGCACCTAAAACAGACG	In this study
clfA-R		TGTGTCGTTTCCTGTTGTGC	
icaA-F	Intercellular adhesion A	AACAGAGGTAAAGCCAACGCACTC	10
icaA-R		CGATAGTATCTGCATCCAAGCAC	
sasG-F	Surface protein G	GGTTTTCAGGTCCTTTTGGAT	10
sasG-R		CTGGTGAAGAGCGAGTGAAA	
spa-F	Staphylococcal protein A	GCGCAACACGATGAAGCTCAACAA	10
spa-R		ACGTTAGCACTTTGGCTTGGATCA	
fnbA-F	Fibronectin binding protein A	ACAAGTTGAAGTGGCACAGCC	11
fnbA-R		CCGCTACATCTGCTGATCTTGTC	
ebpS-F	Elastin binding protein	TTTCCGGTGAACCTGAACCGTAGT	12
ebpS-R		ACAGCAACAACAACGTCAAGGTGG	
sdrD-F	Serine aspartate repeat containing protein	AGTACACAGTGGGAACAGCATC	12
sdrD-R	D	TCTGCAGCCTTTGCTTCTTGGTTC	
Toxin gene			
hla-F	α-haemolysin	AATGAATCCTGTCGCTAATGCCGC	10
hla-R		CTGAAGGCCAGGCTAAACCACTTT	
hld-F	δ-haemolysin	TAATTAAGGAAGGAGTGATTTCAATG	10
hld-R		TTTTTAGTGAATTTGTTCACTGTGTC	
lukE-F	leukotoxin E	GAAATGGGGCGTTACTCAAA	18
lukE-R		GAATGGCCAAATCATTCGTT	
psmα-F	Phenol-soluble modulin-α	ACCCATGTGAAAGACCTCCTTTGT	12
psmα-R		ATGGGTATCATCGCTGGCATC	
sea-F	Enterotoxin A	ATGGTGCTTATTATGGTTATC	17
sea-R		CGTTTCCAAAGGTACTGTATT	
tst-F	Toxic shock syndrome toxin-1	ACCCCTGTTCCCTTATCATC	17
tst-R		AAAAGTGTCAGACCCACTAC	
Exoenzyme gene			
sspA-F	Serine protease A	TGATACACAGCATATCCTCATGCA	13
sspA-R		TGGKCGCGAAGTGCCAATA	
nuc-F	Thermonuclease	ATATGGACGTGGCTTAGCGT	14
nuc-R		TGAATCAGCGTTGTCTTCGCTCCA	
coa-F	Coagulase	AGGTCTTGAAGGTAGCTCAT	15
coa-R		GTTGTATTCACGGATACCTGTA	
aur-F	Aureolysin	TTATATTCTAAGTTCGCWGTCTCTTGTGT	16
aur-R		ATCGGGTGCAAATGACGTAGT	
Regulator gene			
agrA-F	Accessory gene regulator A	TGATAATCCTTATGAGGTGCTT	10
agrA-R		CACTGTGACTCGTAACGAAAA	
arlS-F	Autolysis-related locus S	TGGAATACCAATTCCATGATCT	10
arlS-R		TGCAATCAAATATGATGTGAAGAA	
saeS-F	*S. aureus* exoprotein expression	ATCCGAACAACAAGAAAAAACAG	10
saeS-R		TGATTATACCATCACGTAGTCCTTCA	
rot-F	Repressor of toxin	AAGAGCGTCCTGTTGACGAT	10
rot-R		TTTGCATTGCTGTTGCTCTA	
sigB-F	Sigma factor B	TCAGCGGTTAGTTCATCGCTCACT	10
sigB-R		GTCCTTTGAACGGAAGTTTGAAGCC	
sarA-F	Staphylococcal accessory regulator A	CCTCGCAACTGATAATCCTTATG	10
sarA-R		ACGAATTTCACTGCCTAATTTGA	
16S rRNA-F	Housekeeping gene	CGTGCTACAATGGACAATACAAA	18
16S rRNA-R		ATCTACGATTACTAGCGATTCCA	

clfA: clumping factor A; *icaA*: intercellular adhesion A; *sasG*: surface protein G; *fnbA*: fibronectin binding protein A; *spa*: staphylococcal protein A; *ebpS*: elastin binding protein; *sdrD*: serine aspartate repeat containing protein D; *hla*: α-haemolysin; *lukE*: leukotoxin E; *psmα*: PSM-α; *sea*: enterotoxin A; *tst*: toxic-shock syndrome toxin-1; *hld*: δ-toxin; *sspA*: serine protease A; *nuc*: thermonuclease; *coa*: coagulase; *aur*: aureolysin; *agrA*: accessory gene regulator A; *arlS*: autolysis-related locus S; *saeS*: *S. aureus* exoprotein expression S; *rot*: repressor of toxin; *sigB*: sigma factor B; *sarA*: staphylococcal accessory regulator A. *16S rRNA*: housekeeping gene.

Antibiotics

Tedizolid and linezolid used in the current study were obtained from Cubist Pharmaceuticals (Lexington, MA, USA) and Pfizer (New York, NY, USA), respectively.

Minimal inhibitory concentrations determination

Minimal inhibitory concentrations (MICs) were determined in triplicate using a broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [9]. Briefly, overnight-cultured *S. aureus* colonies were adjusted to 0.5 McFarland turbidity (~1.5 × 10^8 cfu/mL) in sterile saline and seeded in 96-well plates at a final density of approximately 7.5 × 10^5 cfu/mL in the presence of serial drug concentrations. After 20 hours of incubation at 35 °C, the growth inhibition was observed to determine the MIC. *S. aureus* ATCC 29213 was used as the control strain for the susceptibility testing.

Bacterial growth kinetics

Overnight liquid cultures of *S. aureus* strain were diluted 1:100 into 25 mL fresh BHI medium (Oxoid, England, UK) and subcultured at 37 °C with shaking (150 rpm). After 3 hours of incubation (exponential growth phase), antibiotics were added to the cultures at a final concentration of 1/8 MIC, 1/4 MIC, or 1/2 MIC. The cultures were then re-incubated for up to 24 hours, with an untreated control included for comparison. Bacterial growth was monitored hourly by measuring the optical density at 600 nm (OD_{600}) using an ultraviolet spectrophotometer (Unico Instruments, Shanghai, China).

RNA extraction

Cultures were prepared as described above, then incubated for 4 hours at 37 °C following the addition of a subinhibitory concentration (sub-MIC) of antibiotics. The aliquots were centrifuged (12000 rpm, 10 min, 4 °C), and the resulting pellets were incubated (56°C, 1 hour) with TE buffer (10 mM Tris HCl and 1 mM EDTA, pH 8.0) containing lysostaphin (1 mg/mL, Sangon Biotech, Shanghai, China) and proteinase K (20 mg/mL, TaKaRa, Dalian, China) to destroy the cells. The total RNA was then extracted using an RNA Extraction Kit (TaKaRa, Dalian, China) according to the manufacturer's instructions.

cDNA synthesis and qRT-PCR

Bacterial RNA was quantified by a NanoDrop spectrometer (Thermo Fisher Scientific, MA, USA), followed by purifying and reverse transcribing using the PrimeScript™RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, China). Gene expression analysis was performed by quantitative real-time amplification (qRT-PCR) using SYBR Premix Ex TaqTM (TaKaRa, Dalian, China) in a 7500 Real Time PCR System (Applied Biosystems, CA, USA). A total of 23 virulence genes, including 7 adhesion genes (*clfA*, *icaA*, *sasG*, *fnbA*, *spa*, *ebpS,* and *sdrD*), 6 secreted toxin genes (*hla*, *lukE*, *psmα*, *sea*, *tst,* and *hld*), 4 exoenzyme genes (*sspA*, *nuc*, *coa,* and *aur*), and 6 regulator genes (*agrA*, *arlS*, *saeS*, *rot*, *sigB,* and *sarA*), were assessed [10-18]. Primers for qRT-PCR were listed in Table 1. The expression levels of target genes were determined as n-fold changes of *16S rRNA* using the $2^{-\Delta\Delta Ct}$ method in the presence of antibiotics relative to the growth control (untreated group) [18]. All qRT-PCR experiments were carried out in triplicate.

Statistical analysis

One-way analysis of variance (ANOVA) followed by a posteriori Dunnett's test was used for statistical analysis (SAS Institute Inc., NC, USA). The level of statistical significance was $p < 0.05$.

Results

MICs

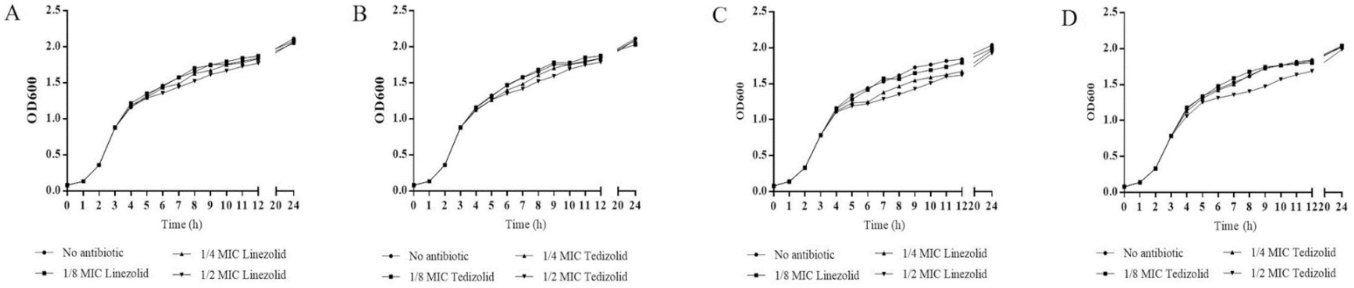
The MIC values of tedizolid and linezolid against two *S. aureus* strains were 0.5/2 μg/mL (strain Newman) and 0.25/1 μg/mL (strain N315), respectively. Based on CLSI breakpoints [9], both

Table 2. Characteristics of *S. aureus* strains used in this study.

S. aureus strain	MIC (μg/mL)		Virulence gene profile																							
			Adhesin gene							Toxin gene						Exoenzyme gene					Regulatory gene					
	Tedizolid	Linezolid	*clfa*	*icaA*	*sasG*	*fnbA*	*spa*	*ebpS*	*sdrD*	*hla*	*lukE*	*psmα*	*sea*	*tst*	*hld*	*sspA*	*nuc*	*coa*	*aur*	*agrA*	*arlS*	*seaS*	*rot*	*sigB*	*sarA*
Newman (MSSA)	0.5, S	2, S	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
N315 (MRSA)	0.25, S	1, S	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+

clfA: clumping factor A; *icaA*: intercellular adhesion A; *sasG*: surface protein G; *fnbA*: fibronectin binding protein A; *spa*: staphylococcal protein A; *ebpS*: elastin binding protein; *sdrD*: serine aspartate repeat containing protein D; *hla*: α-haemolysin; *lukE*: leukotoxin E; *psmα*: PSM-α; *sea*: enterotoxin A; *tst*: toxic-shock syndrome toxin 1; *hld*: δ-toxin; *sspA*: serine protease A; *nuc*: thermonuclease; *coa*: coagulase; *aur*: aureolysin; *agrA*: accessory gene regulator A; *arlS*: autolysis-related locus S; *saeS*: *S. aureus* exoprotein expression S; *rot*: repressor of toxin; *sigB*: sigma factor B; *sarA*: staphylococcal accessory regulator A. MSSA: methicillin-sensitive *S. aureus*; MRSA: methicillin-resistant *S. aureus*; S: susceptibility; -: negative; +: positive.

Figure 1. Influence of sub-MICs of linezolid and tedizolid on growth of *S. aureus*.



Exponentially growing *S. aureus* cultures (3 h incubation) were treated with graded doses of linezolid and tedizolid, and bacterial growth was monitored over time. A and B, strain Newman exposed to varying concentrations of linezolid and tedizolid; C and D, strain N315 treated with different concentrations of linezolid and tedizolid. Bacterial density was measured at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24h. MIC, minimum inhibitory concentration.

strains were susceptible to the oxazolidinones assessed. The characteristics of the two *S. aureus* strains were summarized in Table 2.

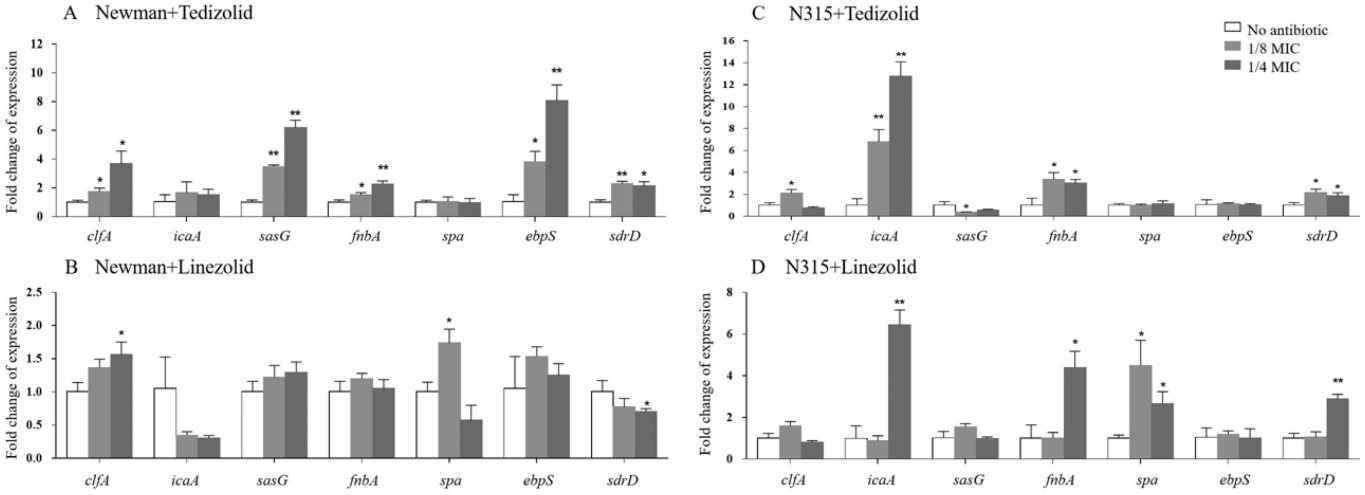
Influence of sub-MICs of oxazolidinones on S. aureus growth

To assess the effects of sub-MICs of linezolid and tedizolid on bacterial growth, we analyzed the growth kinetics of *S. aureus* Newman and N315. As illustrated in Figure 1, exposure to 1/2 MIC of oxazolidinones led to moderate bacterial growth retardation, though cultures reached densities comparable to the control by 24 hours. To minimize potential confounding effects from growth inhibition, we selected 1/4 and 1/8 MICs treatments for evaluating the impacts of subinhibitory antibiotics on *S. aureus* virulence gene expression.

Impacts of sub-MICs of oxazolidinones on the expression of adhesion gene

Treatment with tedizolid significantly modulated the expression of adhesion-related genes in *S. aureus* strains Newman and N315 at sub-inhibitory concentrations (Figure 2). In strain Newman, tedizolid at 1/8 MIC upregulated the expression of genes *clfA* (1.8-fold), *sasG* (3.5-fold), *fnbA* (1.5-fold), *ebpS* (3.8-fold), and *sdrD* (2.3-fold) ($p \leq 0.03$ each). At 1/4 MIC, the upregulated fold changes were 3.7, 6.2, 2.3, 8.1, and 2.1, respectively ($p \leq 0.03$ each). In strain N315, 1/8 MIC tedizolid increased the expression levels of *clfA* (2.1-fold), *icaA* (6.8-fold), *fnbA* (3.4-fold), and *sdrD* (2.2-fold) ($p < 0.03$ each), while inhibiting that of *sasG* by 2.5-fold ($p = 0.03$). At 1/4 MIC, only *icaA* (12.8-fold), *fnbA* (3.0-fold), and *sdrD* (1.9-fold) were markedly upregulated ($p \leq 0.04$ each). Notably, sub-MIC tedizolid did not affect *icaA* and *spa* expression in Newman, nor *spa* and *ebpS* in N315. In contrast, linezolid at 1/4 MIC significantly altered gene expression in a strain-dependent manner: in Newman, it increased *clfA* (1.6-fold) but decreased *sdrD* (1.4-fold); in N315, it upregulated *icaA* (6.5-fold), *fnbA* (4.4-fold), *spa* (2.7-fold), and *sdrD* (2.9-fold). At 1/8 MIC, however, linezolid only notably affected *spa* expression

Figure 2. Effects of tedizolid and linezolid at sub-MICs on adhesion genes expression in *S. aureus* Newman and N315.



Values are means ± SD (n = 3). *, $p < 0.05$ compared to the untreated control. **, $p < 0.01$ compared to the untreated control.

in both strains.

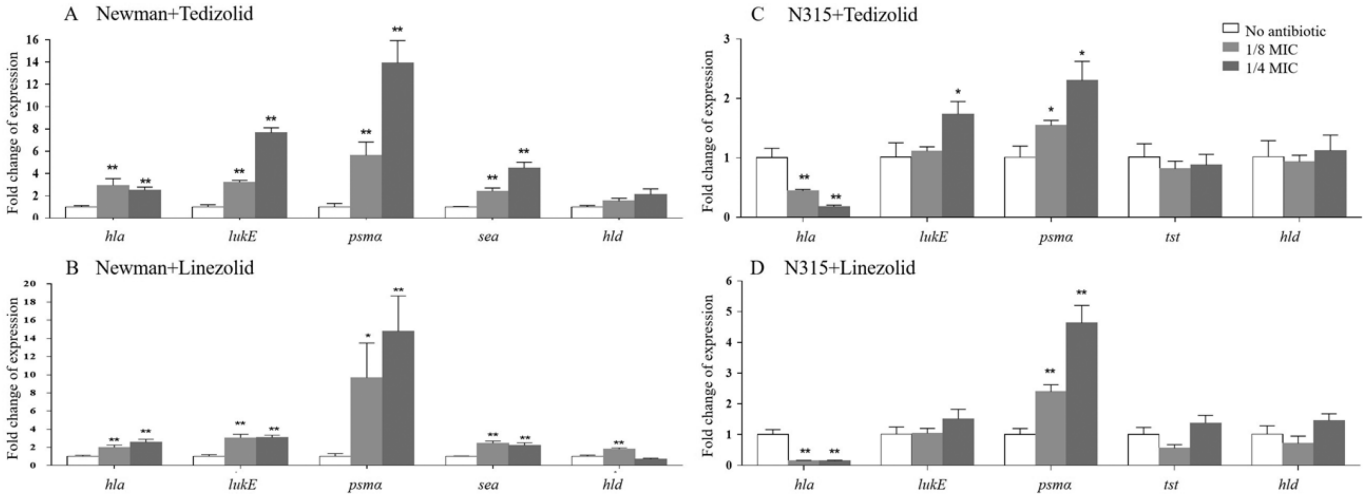
Impacts of sub-MICs of oxazolidinones on toxin gene transcription

Sub-MICs treatment with tedizolid and linezolid (1/8 and 1/4 MIC) significantly upregulated *hla*, *lukE*, *psmα*, and *sea* mRNA levels ($p \leq 0.017$) in strain Newman; notably, 1/8 MIC of linezolid also promoted the expression of *hld* ($p < 0.01$) (Figure 3). In contrast, strain N315 exhibited a more limited response: only the expression of *psmα* was markedly upregulated, while *hla* was significantly suppressed by both drugs at sub-MICs. Interestingly, 1/4 MIC tedizolid also promoted the expression of *lukE* by 1.7 folds ($p = 0.037$) (Figure 3). Neither linezolid nor tedizolid significantly affected the expression of gene *tst* (only exists in strain N315).

Impacts of sub-MICs of oxazolidinones on exoenzyme gene expression

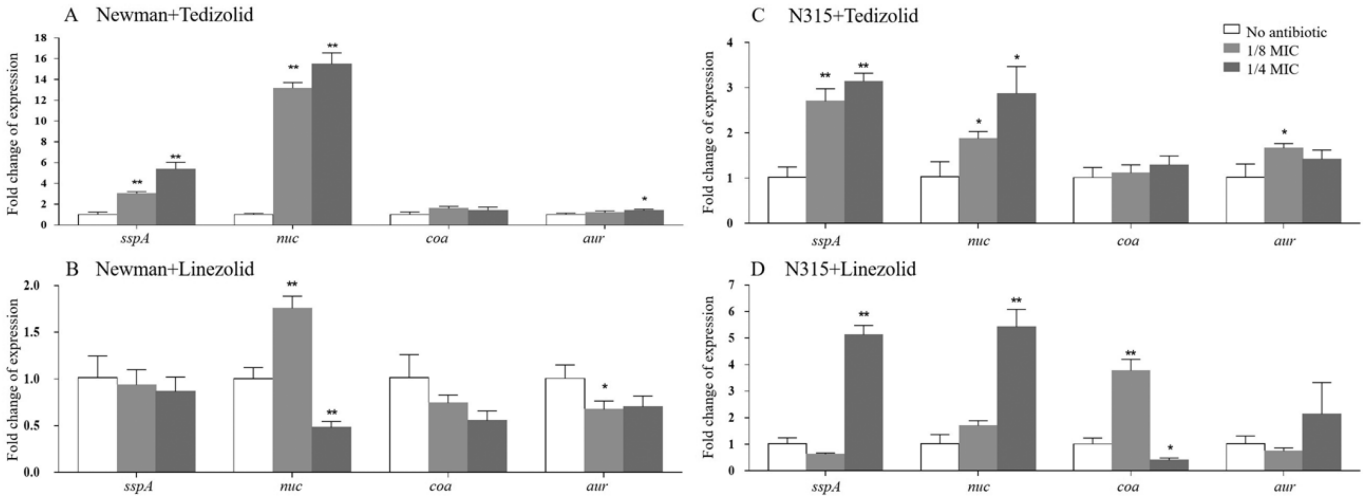
In this section, we compared exoenzyme gene expression before and after treatment with sub-MIC doses of tedizolid and linezolid (Figure 4). The expression levels of several invasive extracellular enzyme genes showed significant variation in both tested strains following treatment with both antibiotics. For instance, tedizolid upregulated three genes—*sspA*, *nuc*, and *aur*—in both Newman (3.0-5.4-fold, 13.2-15.5-fold, and 1.2-1.4-fold, respectively) and N315 (2.7-3.1-fold, 1.9-2.9-fold, and 1.4-1.7-fold,

Figure 3. Impacts of tedizolid and linezolid at sub-MICs on toxin genes expression in *S. aureus* Newman and N315.



Values are means ± SD (n = 3). *, $p < 0.05$ compared to the untreated control. **, $p < 0.01$ compared to the untreated control.

Figure 4. Influences of sub-MICs of tedizolid and linezolid on exoenzyme genes expression in *S. aureus* Newman and N315.



Values are means ± SD (three different experiments). *, $p < 0.05$ compared to the untreated control. **, $p < 0.01$ compared to the untreated control.

respectively) at 1/8-1/4 MICs (Figure 4). Linezolid exhibited a comparable effect: at 1/8 MIC, *nuc* expression increased 1.8-fold in Newman, while *coa* rose 3.8-fold in N315. At 1/4 MIC, N315 showed gene expression upregulation, with *sspA* and *nuc* increasing 5.1- and 5.4-fold, respectively. Conversely, linezolid also led to significant reductions in mRNA levels for genes *nuc* (in Newman at 1/4 MIC), *aur* (in Newman at 1/8 MIC), and *coa* (in N315 at 1/4 MIC) after 4 h of incubation (Figure 4).

Impacts of sub-MICs of oxazolidinones on regulator gene expression

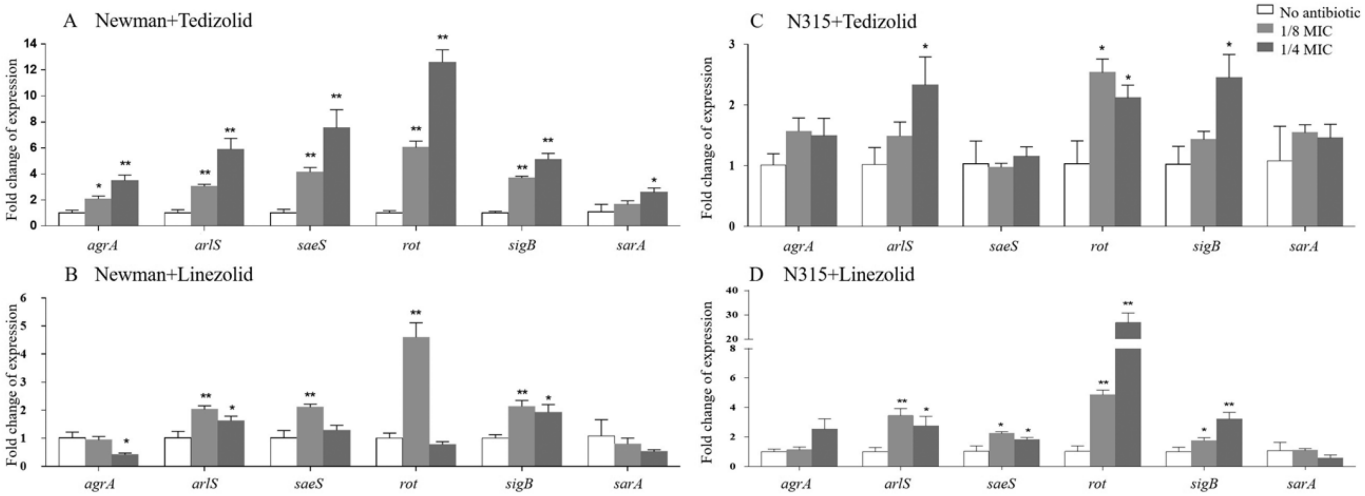
In this section, we examined the impacts of tedizolid and linezolid on 3 two-component regulatory system genes (*agrA*, *arlS,* and *saeS*) and 3 global regulatory genes (*rot*, *sigB,* and *sarA*) (Figure 5). Except for *agrA*, the transcription levels of the studied regulatory determinants were upregulated or unaffected upon drug treatment in both trains. Specifically, treatment with sub-MICs of tedizolid markedly induced all target gene expression in a dose-dependent manner in strain Newman, whereas only 3 genes (*arlS*, *rot,* and *sigB*) expression was induced in strain N315 (demonstrating a strain-dependence) (Figure 5). For linezolid, sub-MIC treatment significantly increased the transcription of *arlS*, *saeS*, *rot,* and *sigB* in both strains; however, it inhibited *agrA* expression in strain Newman, while no significant differences were observed in the expression of *sarA* gene (Figure 5).

Discussion

The clinical effectiveness of antibiotics against *S. aureus* infections depends not only on drug susceptibility but also on their capacity to regulate virulence expression [2,19]. Virulence factors related to adhesion, cell and tissue destruction, and biofilm formation emerge at diverse stages of bacterial infection and remain problematic for clinical management [3]. Some reports indicate that anti-virulence strategies may serve as a promising therapeutic approach in the post-antibiotic era, including the regulation of bacterial virulence by subinhibitory concentrations of antibiotics [2,20]. Previous studies indicated that the second-generation oxazolidinone antibiotic tedizolid is non-inferior to the first-generation oxazolidinone linezolid in treating *Staphylococcus* infection [21,22]. However, the underlying causes of this phenomenon remain unclear. Oxazolidinones are a class of inhibitors of protein synthesis that impede bacterial growth by disrupting the formation of the fMet-tRNA-50S ribosomal subunit. Studies have demonstrated that protein synthesis-inhibitory antibiotics, such as clindamycin and linezolid, can modulate the expression of virulence not only at the translational level but also at the transcriptional level [19,23-25]. We therefore assessed the influence of sub-inhibitory concentrations of both drugs on the transcriptional level of main virulence genes to gain possible insight into the differences between them.

In the current study, we selected 5 genes encoding the cell wall-anchored (CWA) proteins (*clfA*, *fnbA*, *spa*, *sasG,* and *sdrD*) and 2 genes encoding non-covalently surface-associated microbial surface component-recognizing adhesive matrix molecules (MSCRAMMs) (*icaA* and *ebpS*) to evaluate potential differences in the effects of tedizolid and linezolid on *S. aureus* adhesion.

Figure 5. Impacts of tedizolid and linezolid at sub-MICs on regulator genes expression in *S. aureus* Newman and N315.



Values are means ± SD (three different experiments). *, $p < 0.05$ compared to the untreated control. **, $p < 0.01$ compared to the untreated control.

Our data indicated that sub-MIC concentrations of both drugs could enhance the mRNA expression of some virulence genes mentioned above, though this effect varied by strain. Tedizolid increased the expression of *clfA*, *sasG*, *fnbA*, *ebpS,* and *sdrD*, but did not affect the expression of *icaA* and *spa* in strain Newman. In contrast, in strain N315, it increased the transcription of *clfA*, *icaA*, *fnbA,* and *sdrD*, inhibited *sasG*, and did not influence *spa* and *ebpS* mRNA levels (Figure 2). Similarly, while linezolid up-regulated only 2 genes expression in strain Newman, it promoted the transcription levels of 4 genes in strain N315 (Figure 2). In addition, tedizolid and linezolid exerted distinct effects on different genes. Specifically, tedizolid had no impact on *spa* expression, whereas linezolid did not affect the expression of *sasG* and *ebpS* (Figure 2). Notably, an early study demonstrated that sub-MICs of linezolid were capable of reducing *spa* mRNA levels in clinical MRSA strains [19,24]. The divergence from our results may reflect strain-related differences.

The major toxins produced by *S. aureus* include pore-forming toxins (PFTs, such as Hla, LukE, PSM-α, and Hld) and superantigens (SAgs, such as SEA and TSST-1) [26]. Previous studies showed that tedizolid and linezolid suppressed the expression of *hla*, *pvl* (one of the PFTs), and *tst* at the translational level both *in vivo* and *in vitro* [6]. Additionally, Otto *et al.* [19] reported that sub-MICs of linezolid were able to affect the transcriptional level of *hla* in *S. aureus* in a strain-dependent manner. Our data showed that graded sub-MICs of tedizolid promoted the expression of *lukE* and *psmα* in both tested strains. However, its effect on *hla* transcription varied strain-dependently, with upregulation observed in strain Newman and downregulation in strain N315 (Figure 3). Tedizolid increased the expression of *sea* (detected only in strain Newman) but did not affect *tst* (carried by strain N315) (Figure 3). In contrast, linezolid treatment enhanced *psmα* expression in both strains, while its effects on *hla*, *lukE*, and *hld* exhibited strain-dependent variation (Figure 3). Notably, tedizolid and linezolid exerted similar regulatory effects on the toxin genes examined in each strain.

Our experiments showed that tedizolid promoted the expression of *sspA*, *nuc,* and *aur*, but did not affect *coa* expression in both tested strains. In contrast, linezolid exhibited strain-dependent effects, promoting more exoenzyme gene expression in N315 while inducing biphasic regulation of *nuc* in Newman and *coa* in N315. Notably, our findings regarding *coa* expression differed from previous reports showing that sub-MIC linezolid inhibited coagulase production in *S. aureus* [23].

Among microorganisms, two-component systems (TCSs) represent a predominant signal transduction mechanism for sensing and responding to environmental changes [27]. Previous studies have demonstrated that subinhibitory concentrations of antibiotics can regulate the expression of TCS, consequently influencing virulence factor production [28,29]. Our study revealed strain-dependent effects of tedizolid on TCS gene expression: all six examined determinants were upregulated in strain Newman, whereas only three (*arlS*, *rot*, and *sigB*) showed increased expression in strain N315. Notably, linezolid exhibited distinct regulatory effects from tedizolid, upregulating *saeS* in N315 while repressing *agrA* and showing no effect on *sarA* in Newman.

Conclusions

Collectively, our findings demonstrate that protein synthesis inhibitors tedizolid and linezolid differentially regulate the transcription of various virulence genes, with strain-dependent effects observed for specific genes. Notably, both antibiotics showed stimulatory effects on multiple TCS genes, suggesting their potential to modulate virulence factor production by targeting the regulators of complex regulatory networks. Nevertheless, our findings are limited by the unassessed production of virulence factors at the protein level, which represents the final form reflecting the level of virulence. Therefore, future studies should be focused on precisely characterizing the effects of both drugs on bacterial virulence and pathogenicity through functional analyses.

Acknowledgements

We gratefully acknowledge Dr. Shu Jin (People's Hospital of Shanghai Putuo District) for providing the methods used for *Staphylococcus aureus* lysis (Chinese Patent No. ZL201310124795.2).

Authors' contributions

Han Yang and Wen Lin carried out the experiments and wrote the manuscript. Han Yang, Chunyan He, and Wen Shu analyzed the data and interpreted the results. Qingzhong Liu designed the experiments and revised the manuscript. All authors reviewed and approved the manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 82172281, 81772247) and the Natural Science Foundation, Science and Technology Commission of Shanghai (20ZR1444800).

Availability of data and materials

Data supporting the findings of this study are available from the corresponding author on reasonable request.

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Conflict of interest

No conflict of interest is declared.

References

1. Jiang JH, Cameron DR, Nethercott C, Aires-de-Sousa M, Peleg AY (2023) Virulence attributes of successful methicillin-resistant *Staphylococcus aureus* lineages. Clin Microbiol Rev 36: e0014822. doi: 10.1128/cmr.00148-22.
2. Hodille E, Rose W, Diep BA, Goutelle S, Lina G, Dumitrescu O (2017) The role of antibiotics in modulating virulence in *Staphylococcus aureus*. Clin Microbiol Rev 30: 887-917. doi: 10.1128/cmr.00120-16.
3. Chen J, Zhou H, Huang J, Zhang R, Rao X (2021) Virulence alterations in *Staphylococcus aureus* upon treatment with the sub-inhibitory concentrations of antibiotics. J Adv Res 31: 165-175. doi: 10.1016/j.jare.2021.01.008.
4. Miller LG, Flores EA, Launer B, Lee P, Kalkat P, Derrah K, Agrawal S, Schwartz M, Steele G, Kim T, Kuvhenguhwa MS (2023) Safety and tolerability of tedizolid as oral treatment for bone and joint infections. Microbiol Spectr 11: e0128223. doi: 10.1128/spectrum.01282-23.
5. Iqbal K, Milioudi A, Wicha SG (2022) Pharmacokinetics and pharmacodynamics of tedizolid. Clin Pharmacokinet 61: 489-503. doi: 10.1007/s40262-021-01099-7.
6. Yamaki J, Synold T, Wong-Beringer A (2011) Antivirulence potential of TR-700 and clindamycin on clinical isolates of *Staphylococcus aureus* producing phenol-soluble modulins. Antimicrob Agents Chemother 55: 4432-4435. doi: 10.1128/aac.00122-11.
7. Katahira EJ, Davidson SM, Stevens DL, Bolz DD (2019) Subinhibitory concentrations of tedizolid potently inhibit extracellular toxin production by methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. J Med Microbiol 68: 255-262. doi: 10.1099/jmm.0.000905.
8. Le VT, Le HN, Pinheiro MG, Hahn KJ, Dinh ML, Larson KB, Flanagan SD, Badiou C, Lina G, Tkaczyk C, Sellman BR, Diep BA (2017) Effects of tedizolid phosphate on survival outcomes and suppression of production of staphylococcal toxins in a rabbit model of methicillin-resistant *Staphylococcus aureus* necrotizing pneumonia. Antimicrob Agents Chemother 61: e02734-16. doi: 10.1128/aac.02734-16.
9. Wayne PA (2023) Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Susceptibility Testing, 33rd ed.
10. Liu Q, Zheng Z, Kim W, Burgwyn Fuchs B, Mylonakis E (2018) Influence of subinhibitory concentrations of NH125 on biofilm formation & virulence factors of *Staphylococcus aureus*. Future Med Chem 10: 1319-1331. doi: 10.4155/fmc-2017-0286.
11. Balamurugan P, Praveen Krishna V, Bharath D, Lavanya R, Vairaprakash P, Adline Princy S (2017) *Staphylococcus aureus* quorum regulator sara targeted compound, 2-[(methylamino)methyl]phenol inhibits biofilm and down-regulates virulence genes. Front Microbiol 8: 1290. doi: 10.3389/fmicb.2017.01290.
12. Ma Y, Xu Y, Yestrepsky BD, Sorenson RJ, Chen M, Larsen SD, Sun H (2012) Novel inhibitors of *Staphylococcus aureus* virulence gene expression and biofilm formation. PLoS One 7: e47255. doi: 10.1371/journal.pone.0047255.
13. Gustafsson E, Oscarsson J (2008) *Maximal transcription of aur (aureolysin) and sspA (serine protease) in Staphylococcus* aureus requires staphylococcal accessory regulator R (sarR) activity. FEMS Microbiol Lett 284: 158-164. doi: 10.1111/j.1574-6968.2008.01198.x.
14. Olson ME, Nygaard TK, Ackermann L, Watkins RL, Zurek OW, Pallister KB, Griffith S, Kiedrowski MR, Flack CE, Kavanaugh JS, Kreiswirth BN, Horswill AR, Voyich JM (2013) *Staphylococcus aureus* nuclease is an SaeRS-dependent virulence factor. Infect Immun 81: 1316-1324. doi: 10.1128/iai.01242-12.
15. Goerke C, Fluckiger U, Steinhuber A, Bisanzio V, Ulrich M, Bischoff M, Patti JM, Wolz C (2005) Role of *Staphylococcus aureus* global regulators sae and sigmaB in virulence gene expression during device-related infection. Infect Immun 73: 3415-3421. doi: 10.1128/iai.73.6.3415-3421.2005.
16. Korem M, Gov Y, Rosenberg M (2010) Global gene expression in *Staphylococcus aureus* following exposure to alcohol. Microb Pathog 48: 74-84. doi: 10.1016/j.micpath.2009.11.002.
17. Qiu J, Zhang X, Luo M, Li H, Dong J, Wang J, Leng B, Wang X, Feng H, Ren W, Deng X (2011) Subinhibitory concentrations of perilla oil affect the expression of secreted virulence factor genes in *Staphylococcus aureus*. PLoS One 6: e16160. doi: 10.1371/journal.pone.0016160.
18. Yang H, Xu S, Huang K, Xu X, Hu F, He C, Shu W, Wang Z, Gong F, Zhang C, Liu Q (2020) Anti-staphylococcus antibiotics interfere with the transcription of leucocidin ed gene in *Staphylococcus aureus* strain newman. Front Microbiol 11: 265. doi: 10.3389/fmicb.2020.00265.
19. Otto MP, Martin E, Badiou C, Lebrun S, Bes M, Vandenesch F, Etienne J, Lina G, Dumitrescu O (2013) Effects of subinhibitory concentrations of antibiotics on virulence factor expression by community-acquired methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 68: 1524-1532. doi: 10.1093/jac/dkt073.
20. Hao L, Zhou J, Yang H, He C, Shu W, Song H, Liu Q (2024) Anti-virulence potential of iclaprim, a novel folic acid synthesis inhibitor, against *Staphylococcus aureus*. Appl Microbiol Biotechnol 108: 432. doi: 10.1007/s00253-024-13268-2.
21. Roch M, Varela MC, Taglialegna A, Rosato AE (2020) Tedizolid is a promising antimicrobial option for the treatment of *Staphylococcus aureus* infections in cystic fibrosis patients. J Antimicrob Chemother 75: 126-134. doi: 10.1093/jac/dkz418.
22. Carena AA, Stryjewski ME (2020) Tedizolid (torezolid) for the treatment of complicated skin and skin structure infections. Expert Rev Clin Pharmacol 13: 577-592. doi: 10.1080/17512433.2020.1774362.

23. Gemmell CG, Ford CW (2002) Virulence factor expression by Gram-positive cocci exposed to subinhibitory concentrations of linezolid. J Antimicrob Chemother 50: 665-672. doi: 10.1093/jac/dkf192.
24. Bernardo K, Pakulat N, Fleer S, Schnaith A, Utermohlen O, Krut O, Muller S, Kronke M (2004) Subinhibitory concentrations of linezolid reduce *Staphylococcus aureus* virulence factor expression. Antimicrob Agents Chemother 48: 546-555. doi: 10.1128/aac.48.2.546-555.2004.
25. Bonn F, Pané-Farré J, Schlüter R, Schaffer M, Fuchs S, Bernhardt J, Riedel K, Otto A, Völker U, van Dijl JM, Hecker M, Mäder U, Becher D (2016) Global analysis of the impact of linezolid onto virulence factor production in *S. aureus* USA300. Int J Med Microbiol 306: 131-140. doi: 10.1016/j.ijmm.2016.02.004.
26. Oliveira D, Borges A, Simões M (2018) *Staphylococcus aureus* toxins and their molecular activity in infectious diseases. Toxins 10: doi: 10.3390/toxins10060252.
27. Villanueva M, Garcia B, Valle J, Rapun B, Ruiz de Los Mozos I, Solano C, Marti M, Penades JR, Toledo-Arana A, Lasa I (2018) Sensory deprivation in *Staphylococcus aureus*. Nat Commun 9: 523. doi: 10.1038/s41467-018-02949-y.
28. Villanueva M, Roch M, Lasa I, Renzoni A, Kelley WL (2021) The role of ArlRS and VraSR in regulating ceftaroline hypersusceptibility in methicillin-resistant *Staphylococcus aureus*. Antibiotics 10: 821. doi: 10.3390/antibiotics10070821.
29. Liu J, Huang T, Xu Z, Mao Y, Soteyome T, Liu G, Qu C, Yuan L, Ma Q, Zhou F, Seneviratne G (2023) Sub-MIC streptomycin and tetracycline enhanced *Staphylococcus aureus* Guangzhou-SAU749 biofilm formation, an in-depth study on transcriptomics. Biofilm 6: 100156. doi: 10.1016/j.bioflm.2023.100156.