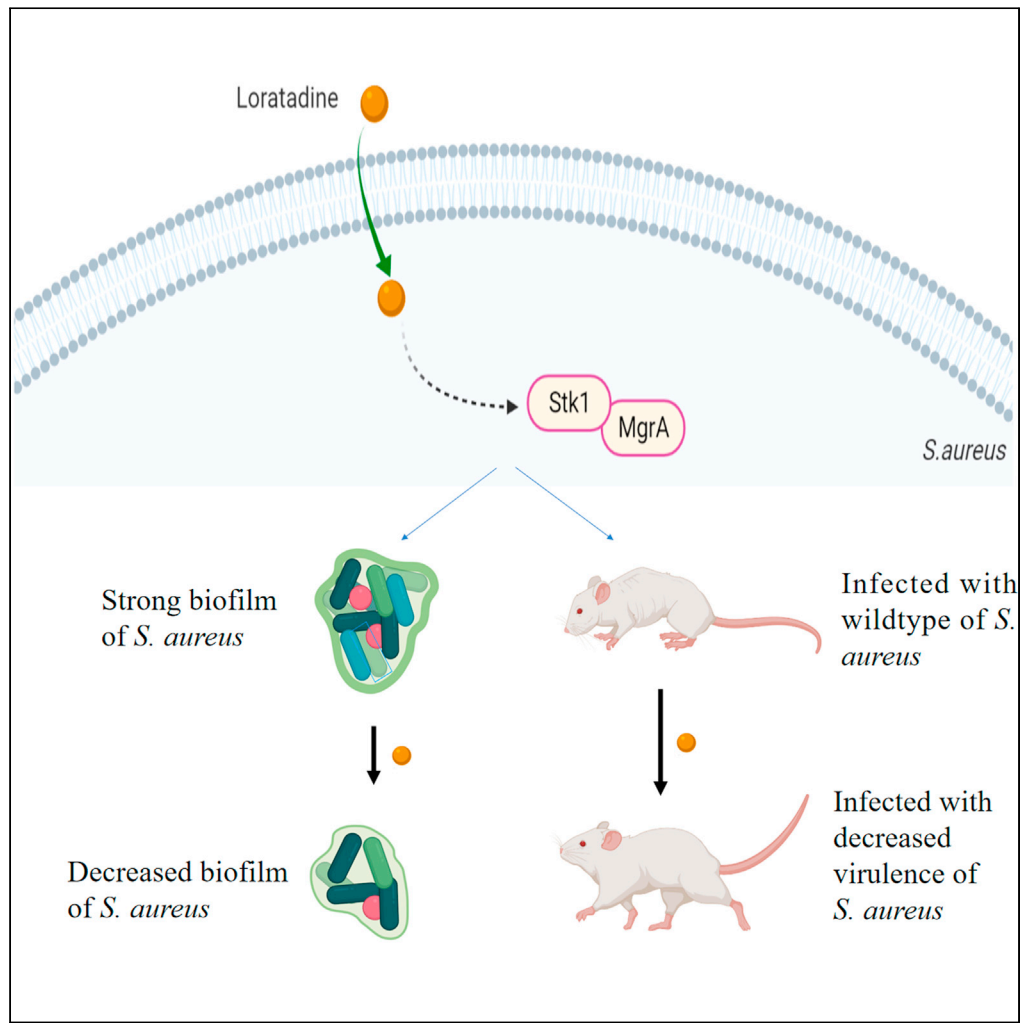


Article

# Loratadine inhibits *Staphylococcus aureus* virulence and biofilm formation



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**Highlights**

Loratadine inhibits *S. aureus* biofilm formation under static or flow conditions

Loratadine reduced mortality in *S. aureus* pulmonary infection model mice

Loratadine synergistically with vancomycin reduced pulmonary bacterial load

Loratadine-induced mutations in MgrA reduced loratadine-MgrA binding



## Article

Loratadine inhibits *Staphylococcus aureus* virulence and biofilm formation

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## SUMMARY

**There are no anti-virulence and anti-biofilm treatments for *Staphylococcus aureus* infection. We found that 25  $\mu$ M loratadine inhibits *S. aureus* biofilm formation under static or flow-based conditions. Testing of loratadine effects on 255 clinical *S. aureus* strains with varying biofilm robustness showed inhibition of biofilm formation in medium and strong, but not weak, biofilm-producing strains. At 25  $\mu$ M, loratadine reduced pigmentation and hemolysis of the bacteria without affecting growth. Loratadine (5 mg/kg) reduced mortality in *S. aureus* pulmonary infection model mice and acted synergistically with vancomycin to reduce pulmonary bacterial load and levels of inflammatory cytokines in bronchoalveolar lavage fluid. Loratadine analogues (side-chain carbamate moiety changed) inhibited biofilm formation, pigmentation, and hemolysis of *S. aureus*. Regarding mechanism, loratadine exposure reduced RNA levels of virulence-related *S. aureus* genes, and loratadine-induced mutations in *MgrA* reduced loratadine-*MgrA* binding. Over-expression of mutated *mgrA* in wild-type *S. aureus* decreased the biofilm formation inhibition effect of loratadine.**

## INTRODUCTION

*Staphylococcus aureus* causes a variety of infectious diseases, from common skin infections such as folliculitis, acne, and granuloma to life-threatening infections such as pneumonia, endocarditis, osteomyelitis, and sepsis. Upon becoming established in its host, *S. aureus* secretes a variety of virulence factors that facilitate its invasion and create disease in the host, including hemolysins, extracellular protease, leukocidin, and phenol-soluble proteins (Tong et al., 2015). Although there are antimicrobials that can clear typical *S. aureus* infections, the emergence of drug-resistant *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA), has resulted in difficult-to-treat cases (Wong et al., 2018). Vancomycin and linezolid are among the few antimicrobials used to treat MRSA infections. However, vancomycin-intermediate *S. aureus* and linezolid-resistant *S. aureus* strains have emerged worldwide in recent years (Katayama et al., 2017; Shariati et al., 2020). Thus, there remains an urgent need for new antimicrobials that can overcome these heartier *S. aureus* strains. Given the slowing of discovery of new antimicrobials in recent years, the strategy of reducing *S. aureus* virulence to decrease its lethality is of great interest (Kong et al., 2016).

In addition to creating the serious problem of drug resistance, *S. aureus* can adhere to the surface of human tissue cells or medical implant materials and then form biofilms (Bhattacharya et al., 2015). In addition to the bacterial cells themselves, *S. aureus* biofilm contains extracellular polysaccharide adhesion molecules, proteins, teichoic acid, and extracellular DNA. The biofilm structure enables chronic infection by protecting *S. aureus* from antimicrobials and host immune-cell phagocytosis (Otto, 2018).

The presence of *S. aureus* biofilms on indwelling medical devices, including artificial heart valves, catheters, and joint prosthetics, has been associated with increased morbidity and longer hospital stay durations, and, ultimately, may lead to infected medical devices needing to be surgically removed (Moormeier and Bayles, 2017). The wide application of various catheters, dialysis technologies, prosthetic joints, and other medical implant materials in modern healthcare settings has led to increasing rates of nosocomial infections with *S. aureus* biofilm (Tasse et al., 2018). The viscous matrix of these biofilms impedes antimicrobial penetration (Verderosa et al., 2019).

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Researchers are working to identify compounds that inhibit *S. aureus* virulence or biofilm formation. Naftifine, cinnamaldehyde, chalcone, streptozotocin, and floxuridine have been shown to reduce the virulence of *S. aureus* (Chen et al., 2016; Ferro et al., 2016; Zhang et al., 2017; Yeo et al., 2018). Meanwhile, boeravinone B, magnolol, ellagic acid, dehydroabiatic acid, and kaempferol have been shown to inhibit *S. aureus* biofilm formation (Singh et al., 2017; Wang et al., 2011; Quave et al., 2012; Fallarero et al., 2013; Ming et al., 2017). A few compounds have been found to both inhibit the virulence and biofilm formation of *S. aureus*. Lee et al. found that the anthraquinone alizarin (10  $\mu\text{g}/\text{mL}$ ) can inhibit *S. aureus* biofilm formation and hemolytic activity (Lee et al., 2016). Subsequently, Baldry et al. found that norlichexanthone can bind the AgrA DNA-binding protein in *S. aureus*; inhibit *S. aureus* biofilm formation by decreasing the expression of *coa*, *hla*, and *RNAIII*; and reduce the toxicity of *S. aureus* to human neutrophils (Baldry et al., 2016). Recently, 2-[(methylamino)methyl]phenol, the benzimidazole derivative UM-C162, myricetin, docosahexaenoic acid, and eicosapentaenoic acid were reported to inhibit *S. aureus* biofilm formation while decreasing *S. aureus* expression of the virulence-related genes *saeR*, *RNAIII*, *fnbA*, and *hla* (Balamurugan et al., 2017; Kong et al., 2018; Silva et al., 2017; Kim et al., 2018). Unfortunately, none of the aforementioned compounds that inhibit *S. aureus* virulence and biofilm formation have been developed into clinically applicable drugs for *S. aureus* infection treatment.

The development of new antimicrobials is generally risky due to being time intensive and costly (Fernandes and Martens, 2017). However, drug development costs can be saved by drug repurposing, which shortens research time and costs by reducing the need for pharmacokinetic and toxicity studies (Savoia, 2016). The aim of this study was to explore compounds already in the US Food and Drug Administration (FDA)-approved drug library for drug repurposing candidates with the ability to inhibit *S. aureus* biofilm formation and virulence.

## RESULTS

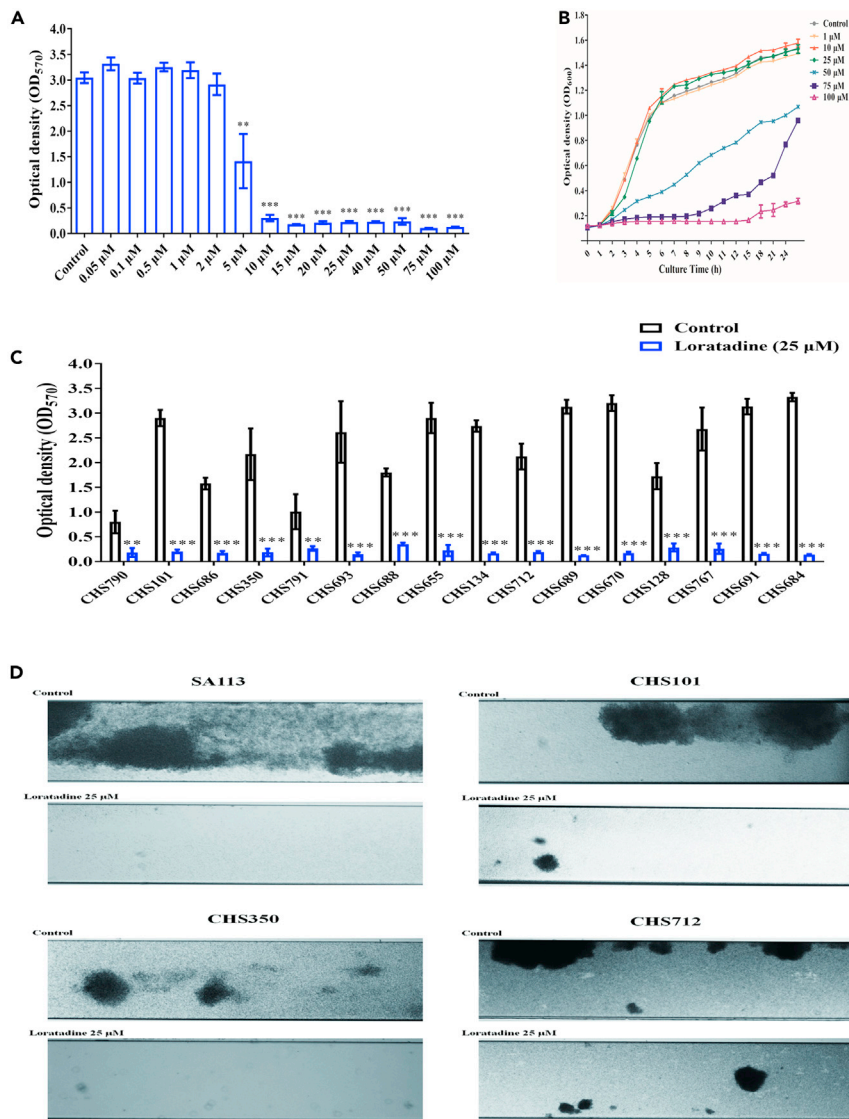
### Loratadine inhibits *S. aureus* biofilm formation

Chemicals in the FDA-approved drug library were screened by checking their effects on crystal violet-stained biofilm samples: *S. aureus* SA113 strain (with chemicals [at 50  $\mu\text{M}$ ] or not) was inoculated into 96-well polystyrene microtiter plates with TSBG (tryptic soy broth with 0.5% glucose). After 24 h of static incubation, the biofilm formed at the bottom of the microtiter plates were measured. The chemical that can significantly inhibit the biofilm formation of *S. aureus* will be focused on and further studied. Exposure to 50  $\mu\text{M}$  loratadine (position: 50101755 rack, F3) for 24 h resulted in significant inhibition of *S. aureus* (SA113 strain) biofilm formation (Table S1 and Figure S1A). In a follow-up experiment examining different concentrations of loratadine, we found that loratadine inhibited *S. aureus* biofilm formation at concentrations  $\geq 5 \mu\text{M}$  (Figure S1B and 1A) but only inhibited growth of *S. aureus* at concentrations  $\geq 50 \mu\text{M}$  (Figure 1B). In a parallel experiment, 25  $\mu\text{M}$  loratadine inhibited biofilm formation of 16 clinical *S. aureus* isolates (including 7 MRSA isolates) (Figure 1C) but did not affect the growth of planktonic *S. aureus* cells from these clinical samples (Figure S2). In flow-based assays performed in a BioFlux 1,000 device, 25  $\mu\text{M}$  loratadine also exhibited a strong inhibitory effect on the biofilm formation of four *S. aureus* strains (Figure 1D).

Interestingly, a comparative assessment of loratadine inhibition of biofilm formation across 255 biofilm-forming clinical *S. aureus* strains (Sun et al., 2018) (including 89 MRSA strains) showed that loratadine inhibited the biofilm formation of medium biofilm producers and strong biofilm producers, while having little effect on weak biofilm producers (Table 1). However, loratadine (1, 10, or 50  $\mu\text{M}$ ) did not disrupt established biofilms of 12 *S. aureus* strains, alone or in combination with antimicrobials (at 8 $\times$  minimum inhibitory concentrations [MICs]) (Table S2, Figure S3 and S4).

### Loratadine reduces *S. aureus* virulence

Although loratadine exposure did not affect growth of planktonic *S. aureus* USA300 (subtype FPR3757) cells at  $\geq 25 \mu\text{M}$  (Figure 2A), it did reduce the pigmentation and hemolysis of the cells (Figures 2B–2D). In an experiment examining the effects of different doses of loratadine on *S. aureus* virulence in a mouse pulmonary infection model, we found that loratadine was most effective at reducing mouse mortality when given at a dose of 5 mg/kg (Figures 2E and S5). Furthermore, co-administration of loratadine and vancomycin had synergistic effects, including effects on reducing *S. aureus* bacterial load in the lungs and reducing levels of inflammatory cytokines (TNF- $\alpha$ , IL-1 $\beta$ , and IL-10) in the bronchoalveolar lavage fluid of model mice (Figure 3).



**Figure 1. Loratadine inhibition of *S. aureus* biofilm formation**

(A) *S. aureus* SA113 was treated, or not, with loratadine (0.05–100 µM, 24 h); 1% crystal violet-stained biofilms were detected by OD<sub>570</sub> analysis.

(B) *S. aureus* SA113 was treated, or not, with loratadine (1–100 µM, 24 h); planktonic cell growth was detected by OD<sub>600</sub> analysis.

(C) Sixteen clinical *S. aureus* isolates were treated with 25 µM loratadine, or not; 1% crystal violet-stained biofilms detected by OD<sub>570</sub> analysis. Data in graphs shown in A, B, and C are means (±standard deviations) of three independent experiments; treated groups were compared with controls; \*\*p < 0.01, \*\*\*p < 0.001 (Student's t test).

(D) Loratadine inhibition of *S. aureus* biofilm formation under a flow condition. Overnight cultures of four *S. aureus* strains were subcultured to a mid-log phase and then diluted 1:200 in TSBG; bacteria were seeded from the outlet well into the channel (shear setting, 2 dyn/cm<sup>2</sup>) for 3 s. After 1-h incubation at 37°C to allow attachment, fresh TSBG containing 25 µM loratadine flowed from the inlet well (shear setting, 0.15 dyn/cm<sup>2</sup>). Bright-field images depicting biofilm development after 16 h were captured at 10 times the original magnification.

### Differentiated effects of loratadine metabolites and analogues on *S. aureus* biofilm formation and virulence

Desloratadine, a biologically active metabolite of loratadine formed by CYP3A4 and CYP2D6 (Bachert, 2009), did not inhibit the biofilm formation of *S. aureus* SA113 (Figures 4A–4C), nor did it reduce the pigmentation and hemolysis of *S. aureus* USA300 (Figures 4D–4F) or reduce mortality in infected mice

**Table 1. Loratadine inhibits biofilm formation by 255 *S. aureus* clinical isolates**

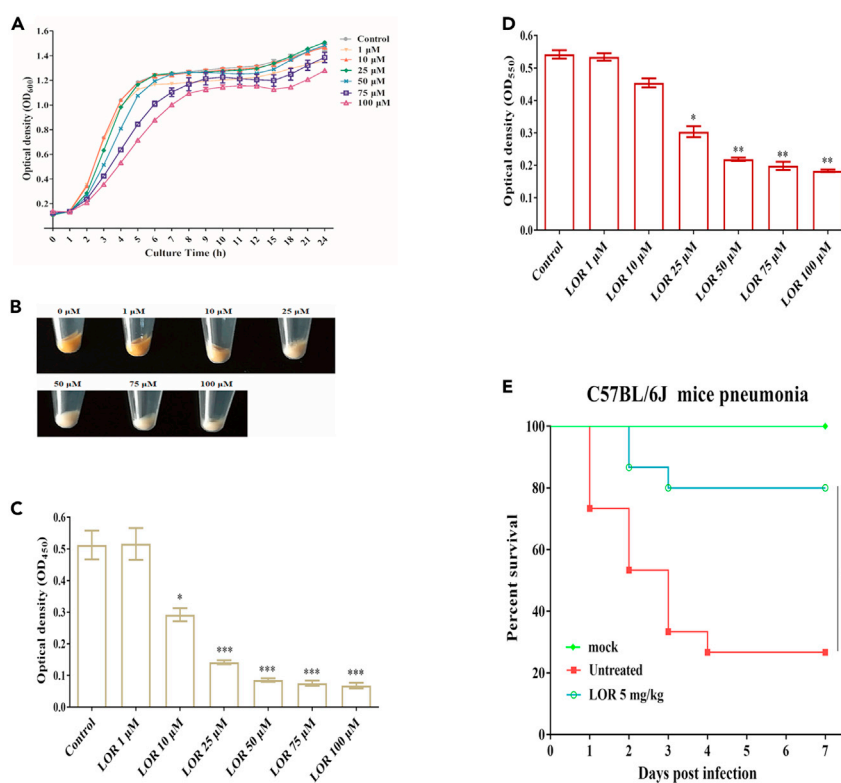
Biofilm-positive isolates	No. (%) of <i>S. aureus</i> isolates with biofilms inhibited by loratadine (25 $\mu$ M)			
	Mild inhibition	Moderate inhibition	Pronounced inhibition	Total inhibition
Weak biofilm producers (n = 130)	8 (6.2)	74 (56.9)	47 (36.1)	129 (99.2)
Medium biofilm producers (n = 88)	0 (0.0)	8 (9.1)	80 (90.9) <sup>a</sup>	88 (100.0)
Strong biofilm producers (n = 37)	0 (0.0)	0 (0.0)	37 (100.0) <sup>b</sup>	37 (100.0)
<b>Total (n = 255)</b>	<b>8 (3.1)</b>	<b>82 (32.2)</b>	<b>164 (64.3)</b>	<b>254 (99.6)</b>

Note: Inhibition rate (%) of biofilm formation (OD<sub>570</sub> of wild type - OD<sub>570</sub> of treated isolate)/OD<sub>570</sub> of wild type. Mild inhibition, 10%  $\leq$  biofilm formation <40% inhibited; moderate inhibition, 40%  $\leq$  biofilm formation <70% inhibited; pronounced inhibition, biofilm formation  $\geq$ 70% inhibited.

<sup>a</sup>Medium versus Weak producers, p < 0.001.

<sup>b</sup>Strong versus Weak producers, p < 0.001 (chi-square test).

(Figure 4G). Because the difference between desloratadine and loratadine is that the former is missing a side-chain carbamate moiety relative to the latter (Ilesce et al., 2019), we hypothesized that the carbamate moiety in the side chain of loratadine may play an important role in antagonizing *S. aureus* biofilm



**Figure 2. Loratadine-induced reduction of *S. aureus* virulence**

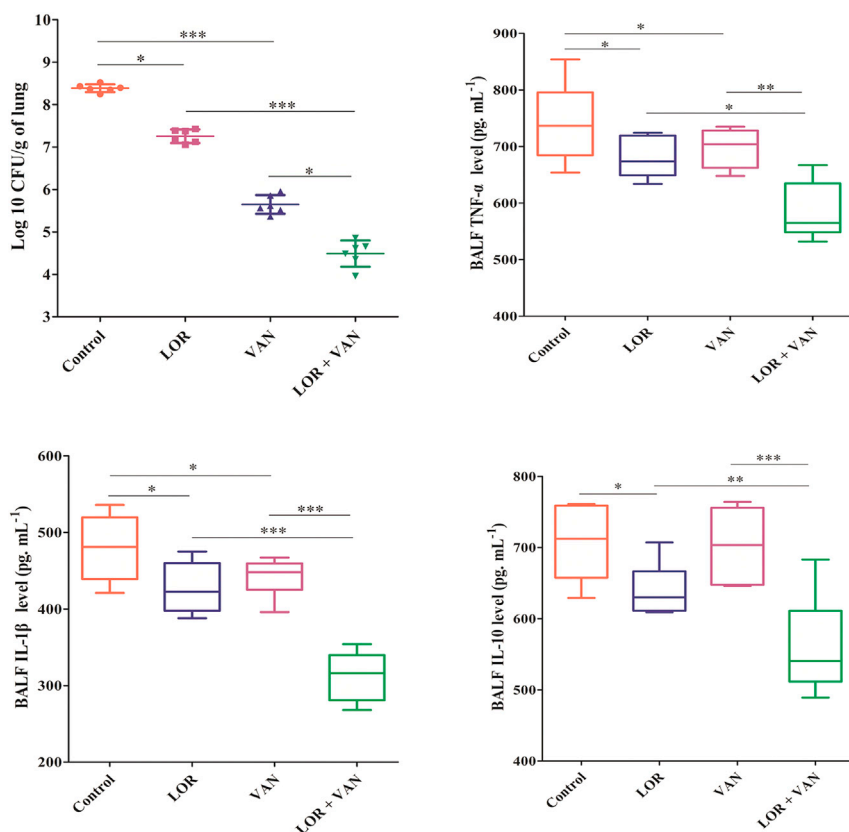
(A) Growth suppressing effects of loratadine on planktonic *S. aureus* USA300 cells, detected by OD<sub>600</sub> analysis.

(B) Images show spun-down cells.

(C) Pigmentation-reducing effects of loratadine on *S. aureus* USA300, detected by OD<sub>450</sub> analysis.

(D) Hemolysis-reducing effect of loratadine on *S. aureus* USA300. Data in graphs shown in A, C, and D are means of three independent experiments ( $\pm$  standard deviations); treated groups were compared with controls; \*p < 0.05, \*\*p < 0.01 (Student's t test).

(E) Protective effect of loratadine (total 5 mg/kg) on survival of mice with *S. aureus* USA300 pulmonary infection (N = 15/ group). The data were collected from two independent experiments; \*\*p < 0.01 (log rank test). LOR, loratadine.



**Figure 3. Loratadine alone or combined with vancomycin decreasing the bacteriology in lung and cytokines in bronchoalveolar lavage fluid of mice**

Female C57BL/6J mice ( $n = 6/\text{group}$ ) suffered from pulmonary infection challenged with *S. aureus* USA300 by nasal drip, loratadine (1 mg/kg) alone or combined with vancomycin (10 mg/kg) were administered to mice by the intraperitoneal injection was started 2 h before the bacterial challenge. After 12 h of bacterial challenge, the C57BL/6J mice were sacrificed and bacteriology in lung and cytokines in bronchoalveolar lavage fluid were determined. The data were collected from two independent experiments and the representative results were shown. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  (Student's t test). LOR, loratadine; VAN, vancomycin; BALF, bronchoalveolar lavage fluid.

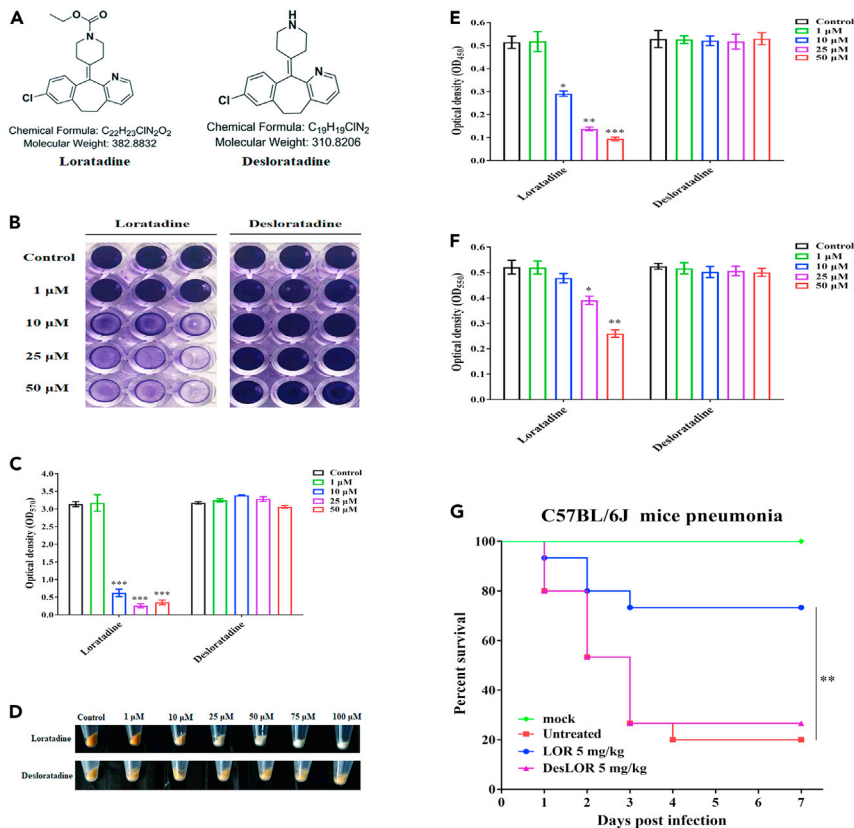
formation and virulence. To test this hypothesis, 10 analogues of loratadine were synthesized with the carbamate moiety in the side chain of loratadine being replaced with other chemical groups (Figures 5A and 5B). These loratadine analogues inhibited *S. aureus* SA113 biofilm formation (Figure 5B) and also reduced pigmentation and hemolysis of *S. aureus* USA300 cells (Figures 5C and 5D).

### Co-localization of loratadine and *S. aureus*

In order to observe the transport of loratadine into *S. aureus* cells, the localization of loratadine and *S. aureus* cells were detected by confocal laser scanning microscope. As can be seen from Figure 6, the DNA of *S. aureus* cells labeled by DAPI (red fluorescence, Figure 6A) completely overlaps with loratadine labeled by FITC (green fluorescence, Figure 6B), and resulted in the overlap yellow image (Figure 6C). This suggested that loratadine was transported into *S. aureus* cells.

### Loratadine reduces RNA levels of *S. aureus* genes related to biofilm formation and virulence

*S. aureus* SA113 or USA300 strains were treated, or not, with 25  $\mu\text{M}$  loratadine and inoculated into the logarithmic phase (4 h); then, total RNA was isolated from the cells and sequenced with an Illumina HiSeq 2500 sequencer. Compared with nontreated controls, this study found that RNA levels of above 800 genes increased or decreased in loratadine-treated *S. aureus* SA113 strains, as shown in Table S3 (RNA levels of genes between the loratadine-treated and untreated of *S. aureus* SA113 strains), and RNA levels of above 600 genes increased or decreased in the loratadine-treated *S. aureus* USA300 strains, as shown in



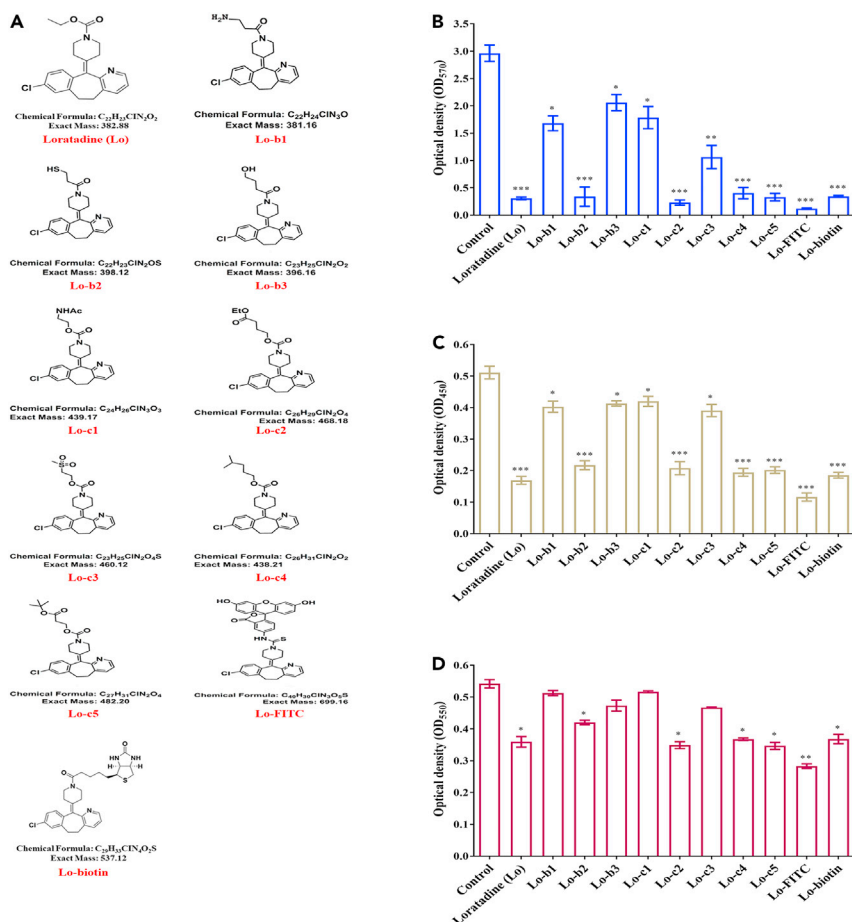
**Figure 4. Different effects of loratadine and desloratadine on *S. aureus* biofilm formation and virulence**

(A–E) Difference of structural formula between loratadine and desloratadine. The *S. aureus* SA113 strain was treated with loratadine or desloratadine, the biofilms were stained with 1% crystal violet (B), and biofilms were detected by optical density at 570 nm ( $OD_{570}$ ) (C). Pigmentation-reducing effects of loratadine or desloratadine on *S. aureus* USA300, images show spun-down cells (D) and detected by optical density at 450 nm ( $OD_{450}$ ) (E). (F) Hemolysis-reducing effects of loratadine or desloratadine on *S. aureus* USA300. Data in graphs shown in C, D, and F are means ( $\pm$  standard deviations) of three independent experiments; treated groups were compared with controls; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  (Student's t test). (G) Effect of loratadine (total 5 mg/kg) or desloratadine (total 5 mg/kg) in protecting female C57BL/6J mice ( $n = 15$ /group) from pulmonary infection challenged with *S. aureus* USA300 by nasal drip. Data were collected from two independent experiments and the representative results were shown. \*\* $p < 0.01$  (log rank test). LOR, loratadine. DesLOR, desloratadine.

**Table S4** (RNA levels of genes between the loratadine treated and the untreated of *S. aureus* USA300 strains). Among these genes, loratadine-treated *S. aureus* cells (SA113 and USA300) had markedly reduced RNA levels of many biofilm formation- and virulence-related genes (Table 2). Quantitative analysis of the RNA levels of these genes indicated that loratadine had an inhibitory influence on biofilm formation and pigmentation of all these isolates. In a follow-up experiment with 25 clinical isolates of *S. aureus* (Table S5 and Figure S7), we found that loratadine treatment reduced the levels of mRNA for the following proteins dramatically in all 25 isolates: serine protease SplB, alpha-hemolysin, gamma-hemolysin HlgCB subunit C, phenol-soluble modulins PSM $\beta$ , and PSM $\delta$ . The RNA levels of the following competence proteins were also reduced significantly by loratadine in most of the 25 clinical isolates: ComK, lipase, thermonuclease, and four transcriptional regulatory genes *agrA*, *saeR*, *sarA*, and *sigB*.

### Genetic mutations in loratadine-induced isolates of *S. aureus*

To explore the potential target of loratadine in *S. aureus*, loratadine non-sensitive isolates were induced *in vitro* and mutations in possible target genes were detected by the whole-genome sequencing. After 180 days of induction (Figure 7A), 23 clones (generation 81 of *S. aureus* SA113) induced under a high concentration (500  $\mu$ M) of loratadine were selected. Crystal violet staining analyses revealed that the biofilm



**Figure 5. Effect of loratadine analogues on *S. aureus* biofilm formation and virulence**

(A) Structural formula of loratadine analogues. Loratadine analogues were designed and synthesized by Xi'an ruixi Biological Technology Co., Ltd (Xi'an, China).  
 (B) *S. aureus* SA113 strain was treated with loratadine or its analogues, and biofilms were stained with 1% crystal violet and were detected by optical density at 570 nm (OD<sub>570</sub>).  
 (C) Effect of loratadine or its analogues on *S. aureus* USA300 pigmentation, detected by optical density at 450 nm (OD<sub>450</sub>).  
 (D) Effect of loratadine or its analogues on *S. aureus* USA300 hemolysis. Data in graphs shown in (B–D) are means ( $\pm$  standard deviations) of three independent experiments; treated groups were compared with controls; \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  (Student's t test).

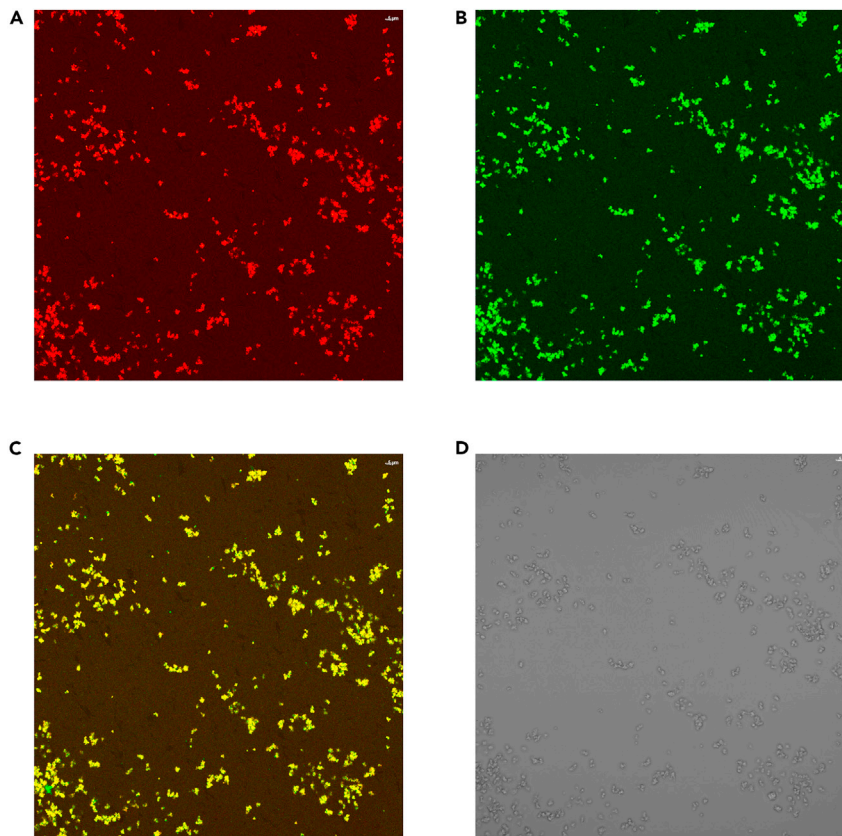
formation ability of two clones (81-1 and 81-6) recovered to levels of the control clone (0-0) and 25  $\mu$ M loratadine did not inhibit their biofilm formation (Figure 7B). In *S. aureus* USA300 strain clones induced under high-concentration (500  $\mu$ M) loratadine, we found that pigmentation and hemolysis were still inhibited by loratadine (data not shown).

Sequencing of the whole genomes of the two aforementioned *S. aureus* SA113 clones (81-1 and 81-6) and of the control clone (serially subcultured in loratadine-free tryptic soy broth [TSB]) with an Illumina HiSeq 2500 sequencer revealed the same genetic mutations affecting five genes in the two clones (Table 3). These mutations led to nonsynonymous mutations of only three amino acids (Table 3). Among the five genes with mutations was the global transcriptional regulator *mgrA*, which has been reported to play an important role in *S. aureus* biofilm formation and virulence (Keinhörster et al., 2019). Thus, we proceeded to examine whether MgrA may be a target of loratadine in *S. aureus*.

### MgrA mutation reduces its loratadine binding affinity

We expressed and purified MgrA protein *in vitro* and analyzed loratadine binding to wild-type versus mutant (R92L) MgrA protein by high-performance liquid chromatography (HPLC)-tandem mass





**Figure 6. Co-localization of loratadine and *S. aureus***

The localization of loratadine and *S. aureus* cells was detected by confocal laser scanning microscope.

(A) The DNA of *S. aureus* cells was labeled by DAPI (red fluorescence).

(B) Loratadine was labeled by FITC (green fluorescence).

(C) Overlay of (A and B)

(D) Phase contrasts.

spectrometry (MS/MS). An HPLC-MS/MS protocol was established for reliable quantification of loratadine-bound MgrA (Figure S8). As shown in Table 4, our HPLC-MS/MS data indicated that loratadine binding of mutant (R92L) MgrA (40,100 ng/mL) was reduced by more than three-quarters relative to loratadine binding of wild-type MgrA (168,000 ng/mL).

### Overexpression of *mgrA* mutation reduces the biofilm formation inhibition effect of loratadine

In order to confirm that *mgrA* gene was involved in loratadine against *S. aureus*, we overexpressed wild-type *mgrA* (*mgrA*-wt) and mutated *mgrA* (*mgrA*-mt, G275T) in *S. aureus* SA113 strain (Figure S9). As indicated in Figure 8, the inhibition effect of loratadine (at 25  $\mu$ M) on *mgrA*-mt overexpression strain (SA113-pRB475-*mgrA*-mt) was reduced compared with that on wild-type control strains (SA113-0-0) or *mgrA*-wt overexpression strain (SA113-pRB475-*mgrA*-wt).

## DISCUSSION

In the present study, we showed that loratadine inhibits *S. aureus* biofilm formation under static or flow-based conditions, with the inhibition being pronounced for strains that are medium to strong biofilm producers. In *S. aureus* pulmonary infection model mice, loratadine reduced mortality and acted synergistically with vancomycin to reduce the bacterial load in the lungs and inflammatory cytokine levels in the bronchoalveolar lavage fluid. Loratadine exposure reduced RNA levels of a variety of genes related to

**Table 2. Differential RNA levels between loratadine treated and non-treated *S. aureus* SA113 and USA300 strains**

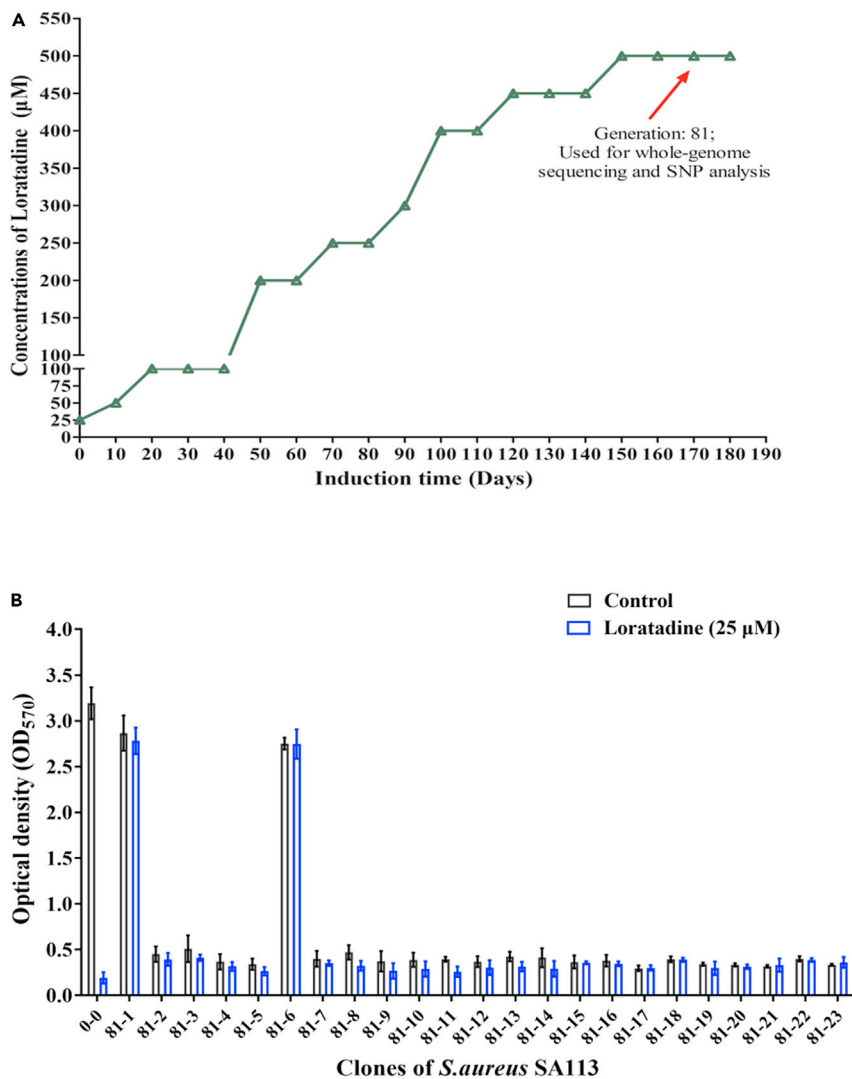
Gene_ID	Description/function	SA113		USA300	
		RNA-seq	RT-qPCR	RNA-seq	RT-qPCR
SACOL_RS09580	Serine protease SplB	0.0010	0.0545	0.0219	0.0175
SACOL_RS05390	Serine protease	0.0414	0.3722	0.1304	0.2344
SACOL_RS06075	Beta-class phenol-soluble modulin (PSM $\beta$ )	0.0160	0.4015	0.0171	0.0115
SACOL_RS10560	Delta-lysin family phenol-soluble modulin (PSM $\delta$ )	0.0336	0.2764	0.0549	0.0461
SACOL_RS06010	Alpha-hemolysin	0.0339	0.0514	0.1058	0.0709
SACOL_RS12705	Bicomponent gamma-hemolysin HlgCB subunit C	0.0311	0.0899	0.1408	0.1843
SACOL_RS06030	Superantigen-like protein SSL13	0.1385	0.4828	0.1161	0.3591
SACOL_RS05265	Competence protein ComK	0.0036	0.3340	0.0105	0.0304
SACOL_RS01600	Lipase	0.0503	0.2481	0.1067	0.1263
SACOL_RS05385	Staphopain B	0.0537	0.3862	0.1173	0.2166
SACOL_RS14075	Poly-beta-1, 6 N-acetyl-D-glucosamine (PNAG) synthase	0.0540	0.0634	0.1130	0.2534
SACOL_RS04415	Thermonuclease	0.0585	0.1522	0.1372	0.0838
SACOL_RS03150	MSCRAMM family adhesin SdrD	0.0950	0.5232	0.1168	0.7497
SACOL_RS01245	DNA-binding response regulator	0.1149	0.8027	0.1037	0.5909
SACOL_RS14015	Serine-rich repeat glycoprotein adhesin SasA	0.1453	0.3009	0.1684	0.4947
SACOL_RS02590	LysR family transcriptional regulator	0.1918	0.5704	0.1106	0.2835
SACOL_RS13890	Clumping factor B (ClfB)	0.3609	1.0099	0.0749	0.4307
SACOL_RS12690	Immunoglobulin-binding protein sbi	0.1168	0.0092	0.1730	0.1531
SACOL_RS10580	DNA-binding response regulator/agrA	0.3077	0.1451	0.4322	0.2060
SACOL_RS03935	Response regulator transcription factor/saeR	0.2425	0.4762	0.4448	0.2259
SACOL_RS03475	Transcriptional regulator/sarA	0.2287	0.3644	0.2180	0.6453
SACOL_RS10765	Serine phosphatase/sigma-B regulation protein	0.4093	0.5231	0.4523	0.3562

**Note:** *S. aureus* strains were inoculated into the logarithmic phase (4 h). Isolated total RNA was sequenced; means of results from three independent experiments were compared. RNA-seq, RNA sequencing. RT-qPCR, reverse transcriptase quantitative polymerase chain reaction. A non-treated isolate was the reference (RNA level = 1.0).

*S. aureus* virulence and biofilm formation. Loratadine-induced mutations in MgrA reduced loratadine-MgrA binding *in vitro*.

Loratadine, a long-acting non-sedating tricyclic antihistamine, was first developed and marketed in 1988 in Belgium by Schering-Plough, a US-based pharmaceutical corporation. It is a second-generation antihistamine that acts as a selective peripheral H1 receptor antagonist. Loratadine differs from first-generation antihistamines in that it has no cholinergic or epinephrine antagonistic effects and has thus far not been found to produce the adverse reactions of central depression or drowsiness (Pasko et al., 2017). It is an effective treatment for a variety of allergic diseases, including asthma, rhinitis, and chronic urticaria (Church and Maurer, 2012; Wei, 2016; Lu et al., 2009), and has also been found to relieve pegfilgrastim-associated bone pain (Pawloski et al., 2016). When taken in combination with tiridazine, loratadine inhibits gastrointestinal cancer cell proliferation by inhibiting phosphatidylinositol 3-kinase/Akt signaling, and loratadine can reduce the expression of inflammatory factors (TNF- $\alpha$ , IL-6, and IL-8), thereby reducing the inflammatory injury of vascular endothelial cells caused by low-density lipoprotein (Chen et al., 2017; Zhou et al., 2018). Cutrona et al. reported that loratadine inhibited biofilm formation in both *S. aureus* and *Staphylococcus epidermidis* and that loratadine potentiated  $\beta$ -lactam antibiotic efficacy against MRSA and potentiated both  $\beta$ -lactam antibiotic and vancomycin efficacy against vancomycin-resistant *S. aureus* (Cutrona et al., 2019).

The virulence of *S. aureus* plays an important role in its pathogenicity. *S. aureus* can harm its host by secreting virulence factors, such as hemolysins, extracellular protease, and leukocidin (Tong et al., 2015). Drug-resistant *S. aureus* strains, including MRSA, represent a serious clinical threat, especially when



**Figure 7. Loratadine non-sensitive clones induced in vitro**

(A) *S. aureus* SA113 strain was serially subcultured in TSB containing loratadine, from the initial inducing concentration at 25 µM and then successively increased to high concentration (500 µM). SNP, single-nucleotide polymorphism.  
(B) The 23 clones from generation 81 of *S. aureus* SA113 strain that were induced under the high concentration (500 µM) of loratadine were selected, and the inhibitory effect of loratadine (25 µM) on biofilm formation of these clones was determined by crystal violet staining. 0-0: the clone from the SA113 control strain, which was serially subcultured in TSB containing no loratadine.

established on implant materials in deep tissues (Hassoun et al., 2017; Inzana et al., 2016). Reducing the virulence of *S. aureus* could thus be helpful for limiting the damage caused by drug-resistant *S. aureus* infections, especially those involving medical implant materials (Ford and Cassat, 2017). With the rising use of medical implants, *S. aureus* biofilm infection on implant materials has been an increasing problem that can bring substantial burden and harm to patients (Tasse et al., 2018). Because it is very difficult to clear an established *S. aureus* biofilm with currently available antimicrobials (Moormeier and Bayles, 2017), it is very important to prevent its formation prophylactically.

The present data indicating that loratadine reduces *S. aureus* virulence and biofilm formation at concentrations  $\geq 25$  µM (9.572 µg/mL) suggest that a typical oral loratadine dosage of a 10-mg tablet once daily, which has been shown to result in a mean plasma  $C_{max}$  of 4.64 ng/mL after 10 days (Kosoglou et al., 2000), is far too low a dose to produce a plasma concentration that would inhibit *S. aureus* virulence and biofilm

**Table 3. Mutations in SA113 strain clones detected by whole-genome sequencing**

Gene_ID	Locus_tag (CP000253.1)	SwissProt	Nucleotide mutation	Amino acid mutation (type)
SA113_GM000528	SAOUHSC_01,012	Q2FI10; Phosphoribosylformylglycinamide synthase subunit PurQ	G563A	G188G (syn)
SA113_GM000613	SAOUHSC_00,920	Q2FI93; 3-oxoacyl-[acyl-carrier-protein] synthase 3	C751T	R251H (nonsyn)
SA113_GM000812	SAOUHSC_00,694	Q2G0B1; HTH-type transcriptional regulator MgrA	G275T	R92L (nonsyn)
SA113_GM001371	SAOUHSC_02,871	Q5HCZ5; Putative acetyltransferase SACOL2570	G412A	G138S (nonsyn)
SA113_GM002521	SAOUHSC_01,877	O35008; Uncharacterized protein YtqA	T786C	I262I (syn)

**Note:** *S. aureus* was serially subcultured in TSB containing 500  $\mu$ M loratadine. Mutations in two clones (81-1, 81-6) from 81 generations (cultured for ~180 days) were detected by whole-genome sequencing. Mutations in the two clones were identical; syn, synonymous mutation; nonsyn, nonsynonymous mutation.

formation. Furthermore, although loratadine had a strong inhibitory effect on *S. aureus* biofilm formation in our experiments, like most other compounds that inhibit biofilm formation, it was not effective for eradicating the established biofilm. Moreover, oral loratadine is absorbed via the gastrointestinal tract and then metabolized to desloratadine by isoenzymes of the cytochrome P450 system in the liver (Keerthana and Vidyavathi, 2018). Similar to a previous study (Cutrona et al., 2019), we found that desloratadine did not affect *S. aureus* biofilm formation. In addition, we found that desloratadine did not decrease *S. aureus* virulence. Thus, given the above-summarized limitations, oral loratadine is not well suited for the treatment of an *S. aureus* biofilm infection, such as endocarditis (Paharik and Horswill, 2016).

Loratadine coating on medical implant materials could be an effective way of increasing local loratadine levels to an effective range. Moreover, when loratadine is coated on the surfaces of implanted materials, it will be present in its active form, rather than as desloratadine, enabling it to combat *S. aureus* virulence and biofilm formation. Studies are needed to test whether loratadine coating on implanted devices would cause any adverse reactions, such as drowsiness.

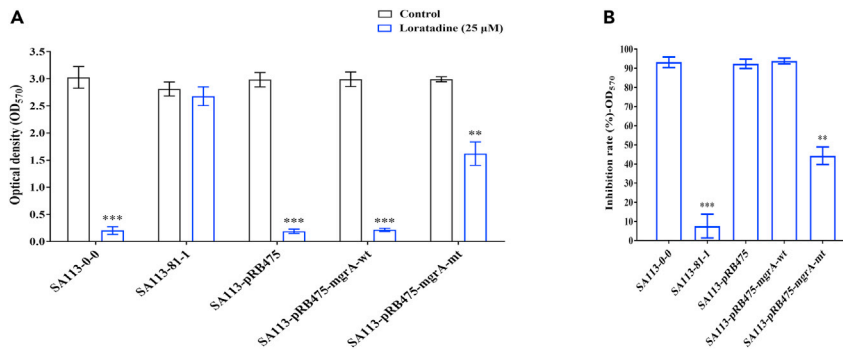
We showed that loratadine can act synergistically with vancomycin to reduce *S. aureus* virulence. Vancomycin is a complex tricyclic glycopeptide antibiotic that inhibits cell wall biosynthesis in gram-positive bacteria (Bruniera et al., 2015). Thus, mechanistically, loratadine likely involves different mechanisms than vancomycin, perhaps interfering with *S. aureus* signal regulation to inhibit virulence and biofilm formation.

Altering a side-chain carbamate moiety that differs between desloratadine and loratadine (Iesce et al., 2019), we found that loratadine analogues maintained the ability to inhibit *S. aureus* biofilm formation and virulence. Precise identification of the target protein of loratadine in *S. aureus*, which has not yet

**Table 4. Loratadine binding of wtMgrA or mtMgrA of *S. aureus***

Sample	Analyte peak area (counts)	Analyte peak height (cps)	Calculated concentration (ng/mL)
LOR- wtMgrA m/z 337.1	1.36E+07	2.07E+06	185,000
LOR- wtMgrA m/z 294	2.33E+07	4.47E+06	176,000
LOR- wtMgrA m/z 267	5.11E+07	8.46E+06	158,000
LOR- wtMgrA m/z 259	3.17E+07	5.67E+06	174,000
LOR- wtMgrA TIC	1.20E+08	2.06E+07	168,000
LOR- mtMgrA m/z 337.1	3.07E+06	5.32E+05	38,000
LOR- mtMgrA m/z 294	5.73E+06	1.01E+06	39,900
LOR- mtMgrA m/z 267	1.42E+07	2.62E+06	40,400
LOR- mtMgrA m/z 259	7.94E+06	1.40E+06	40,100
LOR- mtMgrA TIC	3.10E+07	5.38E+06	40,100

**Note:** LOR, loratadine; wtMgrA, wild-type MgrA; mtMgrA, mutant MgrA (R92L); TIC, total ion chromatogram.



**Figure 8. Effect of loratadine on the biofilm formation of *S. aureus* SA113 with the overexpression of *mgrA*-mt gene**

(A) The *S. aureus* SA113 strains were treated with loratadine 25 μM for 24 h, and the biofilm biomasses were determined by crystal violet staining. The data presented were the average of three independent experiments (mean ± SD). Compared with control: \*\*p < 0.01; \*\*\*p < 0.001 (Student's t test).

(B) Inhibition rate (%) of biofilm formation (the OD<sub>570</sub> value of Control – OD<sub>570</sub> value of the strains treated with loratadine 25 μM/the OD<sub>570</sub> value of Control). Compared with SA113-0-0: \*\*p < 0.01; \*\*\*p < 0.001; (Student's t test). **SA113-0-0**: the clone from the SA113 control strain that was serially subcultured in TSB containing no loratadine; **SA113-81-1**: the clone from the SA113 strain that was serially subcultured in TSB containing loratadine and harboring the *mgrA*-mt (G275T); **SA113-pRB475**: *S. aureus* SA113 with empty plasmid pRB475 as control; **SA113-pRB475-*mgrA*-wt**: SA113 with plasmid pRB475 harboring *mgrA*-wt gene as control for overexpression test; **SA113-pRB475-*mgrA*-mt**: SA113 with plasmid pRB475 harboring *mgrA*-mt gene for overexpression test.

been determined, could enable optimization of the biological activity of loratadine analogues against *S. aureus* virulence and biofilm formation and, thus, reduce the concentration used to achieve efficacy.

Of interest, *S. aureus* virulence- and biofilm formation-related genes whose RNA levels were shown to be reduced by loratadine in this study belong to several signaling pathways. Genes whose mRNA expression levels were suppressed by loratadine included *agr*, *saeRS*, and *sigB*, which are involved in a quorum sensing system, a two-component system, and global stress response regulation, respectively. Based on the above-mentioned activities of loratadine, that is, direct growth inhibition at higher concentration; inhibition of formation and virulence of *S. aureus* by lower sub-inhibitory concentrations; inhibition of expression of numerous virulence factors, regulated by different regulatory systems, as observed by RNA-seq and RT-PCR; observed decreased expression of several regulatory genes like *agrA*, *saeR*, *sarA*, *sigB*; and observations of aforementioned Cutrona et al (2019) that loratadine inhibits Stk1 kinase in *S. aureus*, all indicate that loratadine has nonspecific and wide-ranging activity against numerous regulatory systems of *S. aureus*.

Our whole-genome sequencing data from loratadine non-sensitive isolates revealed *MgrA* mutations, implicating *MgrA* as one of the potential target proteins of loratadine in *S. aureus*. In *S. aureus*, the eukaryotic-like kinase-phosphatase pair Stk1-Stp1 acts to phosphorylate *MgrA* at a cysteine residue (Sun et al., 2012). Previous study indicated that loratadine may inhibit Stk1 (a regulator of penicillin-binding protein and Ser/Thr kinase-associated kinase) in *S. aureus* (Cutrona et al., 2019). Loratadine may target the Stk1-*MgrA* complex to inhibit biofilm formation and virulence of *S. aureus*. In the experiment of loratadine-induced *S. aureus* mutation in this study, *stk1* may be more conservative than *mgrA*, so only *mgrA* mutation was detected. The detailed mechanism of how loratadine affects the regulatory functions of Stk1-*MgrA* remains to be determined.

### Limitations of the study

There are several unsolved issues in this study. First, this study speculated that the way to repurpose loratadine is by using it as an anti-biofilm coating on implants, but this study did not carry out this research, thus new research is needed to explore and verify. Second, this study found that loratadine may target the Stk1-*MgrA* complex to inhibit biofilm formation and virulence of *S. aureus*, but the detailed mechanism of how loratadine affects the regulatory functions of Stk1-*MgrA* remains to be determined. Finally, previous study (Cutrona et al., 2019) and the present study indicated that loratadine had nonspecific and wide-ranging activity against numerous regulatory systems of *S. aureus*; therefore, this means that loratadine may have multiple target proteins or protein complexes in *S. aureus* that need to be further explored.

**STAR★METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**SUPPLEMENTAL INFORMATION**

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.103731>.

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**AUTHOR CONTRIBUTIONS**

J.Z. participated in the design of the study, carried out biofilm biomass analysis and flow-based biofilm detection, analyzed and interpreted the RT-qPCR and whole-genome sequencing data, and drafted the manuscript. Y.S. conducted the hemolysis assay, expression of MgrA, and overexpression of *mgrA*-mt test and participated in whole-genome sequencing data. Y.W. provided Chemicals Screening Libraries (ID: HY-LD-000001025, containing 1,333 chemicals, now as part of the FDA-Approved Drug Library HY-L022) and participated in flow-based biofilm detection and whole-genome sequencing data analysis. Y.Z. participated in the analysis of whole-genome sequencing data and conducted the HPLC-MS assay. Z.C., Z.L., P.L., X.S., and G.X. conducted the antimicrobial susceptibility test, detection of pigment

production, co-localization confocal microscopy imaging, induction of loratadine non-sensitive clones *in vitro*, and whole-genome sequencing. Z.W., J.C., Y.W., Z.W., and Y.X. carried out biofilm biomass analysis, constructed the mice pneumonia model, and carried out quantitation of cytokine levels and measurement of bacteriology in lung in mice. Q.D. participated in analysis of RT-qPCR and whole-genome sequencing data. D.Q. and Z.Y. designed the study, participated in the data analysis, and provided critical revisions of the manuscript for valuable intellectual content.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

## INCLUSION AND DIVERSITY

We worked to ensure diversity in experimental samples through the selection of the genomic datasets. While citing references scientifically relevant for this work, we also actively worked to promote gender balance in our reference list.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Experimental models: organisms</b>		
C57BL/6J mice	Shanghai Jiesijie Laboratory Animal Co., Ltd (Shanghai, China)	N/A
<b>Bacterial strains</b>		
<i>Staphylococcus aureus</i>	ATCC	ATCC 29213
<i>Staphylococcus aureus</i>	ATCC	ATCC 35556
<i>Staphylococcus aureus</i>	ATCC	ATCC BAA-1556
<i>Staphylococcus aureus</i>	the 6th Affiliated Hospital of Shenzhen University Health Science Center	clinical isolates
<i>Escherichia coli</i>	TaKaRa Biotechnology, Dalian, China	DC10B
<b>Chemicals, peptides, and recombinant proteins</b>		
Oxacillin sodium	MedChemExpress	HY-B0465; CAS: 7240-38-2
Cefazolin sodium	MedChemExpress	HY-B1078; CAS: 27164-46-1
Vancomycin	MedChemExpress	HY-B0671; CAS: 1404-90-6
Linezolid	MedChemExpress	HY-10394; CAS: 165800-03-3
Daptomycin	MedChemExpress	HY-B0108; CAS: 103060-53-3
Rifampin	MedChemExpress	HY-B0272; CAS: 13292-46-1
Minocycline hydrochloride	MedChemExpress	HY-17412; CAS: 13614-98-7
Azithromycin	MedChemExpress	HY-17506; CAS: 83905-01-5
Clindamycin	MedChemExpress	HY-B0408; CAS: 21462-39-5
Loratadine	MedChemExpress	HY-17043; CAS: 79794-75-5
Desloratadine	MedChemExpress	HY-B0539; CAS: 100643-71-8
Chemicals Screening Library	MedChemExpress	HY-LD-000001025
Loratadine analogues Lo-b1 to b3, Lo-c1 to c5, Lo-FITC, and Lo-biotin	Xi'an Ruixi Biological Technology Co., Ltd (Xi'an, China).	N/A
DAPI	Sigma Aldrich	D9542; CAS: 28718-90-3
<b>Critical commercial assays</b>		
Mouse Tumor Necrosis Factor $\alpha$ (TNF- $\alpha$ ) elisa kit	Andy Gene, Beijing, China	AD3051Mo
Mouse Interleukin 1 $\beta$ (IL-1 $\beta$ ) elisa kit	Andy Gene, Beijing, China	AD3364Mo
Mouse interleukin 10 (IL-10) elisa kit	Andy Gene, Beijing, China	AD2837Mo
RNeasy minikit	Qiagen, Hilden, Germany	74104
DNeasy Blood & Tissue Kit	Qiagen, Hilden, Germany	69506
mRNA-seq sample preparation kit	Illumina	20020594
NEBNext® Ultra™ DNA Library Prep Kit	NEB	Catalog #: E7370S
SYBR Premix Ex Taq II Kit	TaKaRa Biotechnology, Dalian, China	RR390W
<b>Deposited data</b>		
Raw RNA-Seq data	This paper	Sequence Read Archive (SRA) database : PRJNA587170
Raw whole-genome sequencing data	This paper	Sequence Read Archive (SRA) database : PRJNA649959
<i>S. aureus</i> COL reference genome	Genome Reference	<a href="https://www.ncbi.nlm.nih.gov/nucleotide/CP000046.1?report=graph">https://www.ncbi.nlm.nih.gov/nucleotide/CP000046.1?report=graph</a>

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**Continued**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<i>S. aureus</i> NCTC 8325 reference genome	Genome Reference	<a href="https://www.ncbi.nlm.nih.gov/nucleotide/NZ_L5483365.1?report=graph">https://www.ncbi.nlm.nih.gov/nucleotide/NZ_L5483365.1?report=graph</a>
<b>Oligonucleotides</b>		
Primer: PCR amplification of Full-length <i>mgrA</i> ; forward, GACTGGTGGACAGCAAATGGGT CGCGGATCCATGTCTGATCAACA TAATTTAAAGAAGACAGCTATGCTTT AG; reverse, GATCTCAGTGGTGGTG GTGGTGGTCTCGAGTTATTTTCC TTTGTTTCATCAAATGCATGAATGACTT.	This paper	N/A
Primers for overexpression of <i>mgrA</i> gene in wildtype <i>S. aureus</i> , see <a href="#">Tables S7</a> and <a href="#">Table S8</a>	This paper	N/A
<b>Recombinant DNA</b>		
Plasmid: pRB475;	<a href="#">Bai et al. (2019)</a>	a shuttle plasmid, Km <sup>r</sup> in <i>E. coli</i> and Cm <sup>r</sup> in <i>S. aureus</i>
Plasmid: pRB475- <i>mgrA</i> -wt;	This paper	a shuttle plasmid, Km <sup>r</sup> in <i>E. coli</i> and Cm <sup>r</sup> in <i>S. aureus</i> , the <i>mgrA</i> -wt gene was cloned into plasmid pRB475
Plasmid: pRB475- <i>mgrA</i> -mt;	This paper	a shuttle plasmid, Km <sup>r</sup> in <i>E. coli</i> and Cm <sup>r</sup> in <i>S. aureus</i> , the <i>mgrA</i> -mt gene was cloned into plasmid pRB475
<b>Software and algorithms</b>		
IMARIS 7.0.0	Bitplane, Zurich, Switzerland	N/A
Bowtie2 software (version 2.0.5)	NCBI website	N/A
RepeatMasker	<a href="#">Tempel (2012)</a>	<a href="http://www.repeatmasker.org/">http://www.repeatmasker.org/</a>
Prism 5.01	GraphPad Software, La Jolla, CA	N/A

**RESOURCE AVAILABILITY****Lead contact**

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Zhijian Yu ([yuzhijiansmu@163.com](mailto:yuzhijiansmu@163.com)).

**Materials availability**

The study did not generate any unique reagents.

**Data and code availability**

- This published article includes all datasets generated or analyzed during this study.
- The raw RNA-Seq data was posted in the Sequence Read Archive (SRA) database under accession number PRJNA587170 (<https://dataview.ncbi.nlm.nih.gov/object/PRJNA587170>). The raw whole-genome sequencing data was posted in the Sequence Read Archive (SRA) database under accession number PRJNA649959 (<https://dataview.ncbi.nlm.nih.gov/object/PRJNA649959>).
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

**EXPERIMENTAL MODEL AND SUBJECT DETAILS**

- *S. aureus* strains ATCC29213, SA113 (ATCC35556), and USA300 FPR3757 (ATCC BAA-1556) were purchased from American Type Culture Collection. There were 271 *S. aureus* clinical isolates collected from the 6th Affiliated Hospital of Shenzhen University Health Science Center. This was approved by the ethics committee of 6th Affiliated Hospital of Shenzhen University Health Science Center, in accordance with the ethical standards of Shenzhen University and the 1964 Helsinki declaration and its later amendments, or comparable ethical standards.

- All animal experiments were carried out in accordance with the guidelines of, and was approved by the Committee of Animal Ethics of the Shanghai Medical College of Fudan University. **Murine pneumonia model:** Six-week-old female C57BL/6J mice were anesthetized with sodium pentobarbital and infected via nasal drops with 20  $\mu$ L of the *S. aureus* USA300 suspension ( $1.5 \times 10^{10}$  colony forming units/mL). Treatments, administered via 100- $\mu$ L intraperitoneal injections, were started 2 h before the bacterial challenge. Infected mice were monitored for morbidity over 7 d and prepared for quantitative bacteriology of the lungs and cytokine assays. Statistical analyses were done in comparison to the control group.

## METHOD DETAILS

### Bacterial strains and growth conditions

*S. aureus* ATCC29213, SA113 (ATCC35556), and USA300 FPR3757 (ATCC BAA-1556) strains were purchased from American Type Culture Collection. We also used 271 *S. aureus* clinical isolates collected from the 6th Affiliated Hospital of Shenzhen University Health Science Center (Grade A, level III Hospital, 1500 beds) between January 1, 2014 and December 31, 2018. All clinical isolates and oxacillin sensitivity were identified with a Phoenix 100 automated microbiology system (BD, Franklin Lakes, NJ, USA) and then two subcultured generations were re-identified with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (IVD MALDI Biotyper, Germany). *S. aureus* was grown in TSB at 37°C with shaking unless otherwise stated. For antimicrobial susceptibility testing, *S. aureus* was grown in cation-adjusted Mueller-Hinton broth at 37°C with shaking. For biofilm assays, *S. aureus* was grown in TSB with 0.5% glucose (TSBG) at 37°C under static incubation.

### Antimicrobials and chemicals

Oxacillin sodium (HY-B0465), ceftazolin sodium (HY-B1078), vancomycin (HY-B0671), linezolid (HY-10394), daptomycin (HY-B0108), rifampin (HY-B0272), minocycline hydrochloride (HY-17412), azithromycin (HY-17506), clindamycin (HY-B0408), loratadine (HY-17043), desloratadine (HY-B0539), and Chemicals Screening Libraries (HY-LD-000001025; 1,333 chemicals, part of the FDA-Approved Drug Library) were purchased from MedChemExpress (MCE, Shanghai, China). Loratadine analogues Lo-b1 to b3, Lo-c1 to c5, Lo-FITC, and Lo-biotin were designed and synthesized at Xi'an Ruixi Biological Technology Co., Ltd (Xi'an, China). Media were supplemented with 50 mg/L  $\text{Ca}^{2+}$  for *in vitro* daptomycin testing.

### Antimicrobial susceptibility testing

Antimicrobial MICs were determined by the broth macrodilution method in cation-adjusted Mueller-Hinton broth according to Clinical and Laboratory Standards Institute guidelines (CLSI-M100-S27). Antimicrobial susceptibility results were confirmed based on CLSI-M100-S27. All experiments were performed in triplicate.

### Biofilm biomass determined by crystal violet staining

**Crystal violet staining.** *S. aureus* after static incubation at 37°C, the plates were washed gently three times with phosphate-buffered saline (200  $\mu$ L/well) to remove unattached bacteria, dried at room temperature and the cells fixed with methanol for 15 min. The methanol was removed and cells were stained with 0.5% (100  $\mu$ L/well) crystal violet (CV) for 10 min at room temperature. The optical density at 570 nm (OD570) was determined. Biofilm formation of *S. aureus* was inhibited: *S. aureus* was inoculated into 96 polystyrene microtiter plates with TSBG (with or without compounds). After 24 h of static incubation, biofilms were visualized with 1% crystal violet staining. Established biofilms were eradicated: *S. aureus* was inoculated onto 96 polystyrene microtiter plates. After 24 h of static incubation at 37°C (mature biofilms formed), supernatants were discarded, the plates were washed with 0.9% saline to remove unattached cells, and fresh TSBG (with or without compounds) was added. After 48h of static incubation with the medium replaced daily, biofilms were assessed with crystal violet staining. All experiments were performed in triplicate at least three times.

### Flow-based biofilm detection by bioflux

Microfluidic channels of BioFlux 48-well plates (Fluxion Biosciences, South San Francisco, CA) were primed with pre-warmed TSB medium. Overnight cultures of *S. aureus* were subcultured to mid-log phase and then diluted 1:200 in TSBG medium. The bacteria were seeded from the outlet well into the channel and viewing window at a shear setting of 2  $\text{dyn}/\text{cm}^2$  for 3 s. After a 1-h incubation at 37°C to allow bacteria attachment, fresh TSBG medium containing loratadine (25  $\mu$ M) was set to flow from the inlet well at a shear setting of

0.15 dyn/cm<sup>2</sup>. Biofilm growth was monitored for up to 16 h. Images of growth stages were acquired automatically at 10-min intervals under bright-field illumination.

### Detection of pigment production

Overnight *S. aureus* cultures were diluted 1:200 in TSB medium (with or without compounds), then cultured at 37°C for 48 h with shaking. Subsequently, 4-mL bacteria cultures were centrifuged and washed twice with 0.01 M phosphate-buffered saline (PBS). Pigment was extracted thrice with methanol and added to a total volume of 1 mL. Optical density was determined at 450 nm (OD<sub>450</sub>). The experiments were performed independently in triplicate.

### Hemolysis assay

Overnight *S. aureus* cultures were diluted 1:200 in fresh TSB medium and compounds were added to the medium. After 16 h at 37°C with shaking, bacteria were collected from cultures and adjusted to an OD<sub>600</sub> of 2.0. Bacterial samples (4 mL) were centrifuged (4000 ×g, 4°C, 10 min), the supernatants were filtered with a 0.22-μm filter, and 500-μL supernatant samples were mixed with 500-μL defibrinated rabbit blood. After incubation of the supernatant-blood mixture samples at 37°C for 10 min, the samples were centrifuged (4000 ×g, room temperature, 10 min) and their OD<sub>550</sub> values were determined. Triton X-100 was used as a positive control, and 0.01 M PBS was used as a negative control. Each test was performed independently in triplicate.

### Murine pneumonia model

Six-week-old female C57BL/6J mice (18–20 g) were purchased from Shanghai Jiesijie Laboratory Animal Co., Ltd (Shanghai, China). They were given food and water *ad libitum* for 7 d before inoculation. Mice were treated in accordance with institutional policies and the guidelines stipulated by the animal welfare committee. The Committee of Animal Ethics of the Shanghai Medical College of Fudan University approved all animal experimentation in this study.

Overnight *S. aureus* USA300 (subtype FPR3757) cultures were inoculated 1:100 into fresh MHB and grown at 37°C with shaking for 3 h. Bacteria cultures were sedimented, washed, and suspended in sterile PBS to obtain an inoculum of 1.5 × 10<sup>10</sup> colony forming units (CFU)/ml. The mice were anesthetized with sodium pentobarbital and infected via nasal drops with 20 μL of the bacterial suspension. Treatments, administered via 100-μL intraperitoneal injections, were started 2 h before the bacterial challenge. Infected mice were monitored for morbidity over 7 d and prepared for quantitative bacteriology of the lungs and cytokine assays, described below.

### Quantitation of cytokine levels and lung bacteriology

After a 12-h bacterial challenge, the C57BL/6J mice were sacrificed and bronchoalveolar lavage fluid was collected and centrifuged at 2000× g at 4°C for 10 min. The supernatant was stored at –80°C until testing. TNF-α (tumor necrosis factor α), IL-1β (interleukin 1β), and IL-10 (interleukin 10) concentrations in the bronchoalveolar lavage fluid of mice were quantified with ELISA kits according to the manufacturer instructions (Andy Gene, Beijing, China). Mouse lungs were removed, weighed, homogenized in 500 μL of sterile PBS, ten-fold diluted, and plated on tryptic soy agar. CFU quantities were determined after the dilutes homogenates were in cultures at 37°C for 48 h. Bacterial presence in lungs are expressed as mean (± standard deviation)log<sub>10</sub> CFU/g. These experiments were performed independently at least twice.

### Co-localization confocal microscopy imaging

The co-localization of loratadine and *S. aureus* was performed by the confocal microscopy study. Overnight *S. aureus* SA113 cultures were inoculated 1:100 into 1 mL of TSBG containing 50 μM Lo-FITC (Loratadine-FITC) in FluoroDishes (FD35-100; WPI, Sarasota, FL, United States) and incubated statically for 24 h. Planktonic cells were removed and washed three times with saline. DAPI (Sigma Aldrich) was used to stain DNA at a final concentration of 5 μg/mL. Experiments were performed using a confocal laser scanning microscope (TCS-SP5, Leica, Wetzlar, Germany) with a 40× air immersion objective. The images were analyzed by IMARIS 7.0.0 software package (Bitplane, Zurich, Switzerland).

### RNA isolation and RNA sequencing

The planktonic *S. aureus* cells were homogenized by the 0.1-mm zirconia-silica beads in a mini-Bead Beater and then purified total RNA in the supernatant using an RNeasy minikit (Qiagen, Hilden, Germany).



and then cloned into pET-28a plasmids. Correct recombinant plasmids were identified by restriction enzyme digestion and sequencing, and then transferred into *Escherichia coli* BL21 (DE3) competent cells. Expression of MgrA was induced with 1 mM isopropyl- $\beta$ -D-thiogalactoside. The product was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and western blot assays. MgrA protein was purified by Ni column affinity chromatography.

### Ultrafiltration by HPLC-MS/MS

Loratadine solution (1  $\mu$ L, 1 mM) was incubated at 37°C for 0.5 h with 100  $\mu$ L of 0.5  $\mu$ M MgrA or mutant MgrA (R92L) in PBS. Each mixture was then subjected to centrifugal separation ultrafiltration (10KD cellulose ultrafiltration membrane; Pall Corporation, Nanosep with 10K Omega, Fajardo, Puerto Rico). To remove the unbound compounds, the filter was washed three times with 0.1 M ammonium bicarbon. The bound ligands were released with methanol/water (50:50 vol/vol; pH 3.30) and centrifuged. The released ligands and calibration curve solution (250 to 40000 ng/mL) were submitted to LC-MS/MS system (Sciex 4500). HPLC separations were carried out on a C18 column (2.1  $\times$  50 mm, 3.5 $\mu$ m, Water XBridge). The flow rate was set to 0.3 mL/min and the eluting gradient was composed of water (A) and acetonitrile (B): the elution started with 10% B for the first 0.5 min, followed by a linear gradient from 10% B to 90% B over the next 4.5 min, maintenance at 90% B for another 3 min, and 10% B for another 1 min. The mass spectrometer was operated in the positive ion mode with default values. Compound parameters (including Q1 Scan and product Ion Scan) were preset at recommended/default values. Quantitative data were analyzed in Analyst 1.6.

### Overexpression of mgrA gene in wildtype *S. aureus*

The full-length genes with their promoter region of wildtype mgrA (*mgrA*-wt) and mutated mgrA (*mgrA*-mt, G275T) were amplified by PCR, the amplicons were purified and digested with endonucleases, and then cloned into pRB475 plasmid for gene overexpression. The pRB475, pRB475-*mgrA*-wt, pRB475-*mgrA*-mt plasmids were transferred into the *S. aureus* SA113 strain by electroporation. All strains, plasmids, and primers used for overexpression analysis were listed in [Table S7](#) (Iordanescu and Surdeanu, 1976; Monk et al., 2012; Bai et al., 2019) and [Table S8](#).

### QUANTIFICATION AND STATISTICAL ANALYSIS

The data were visualized in Prism 5.01 software (GraphPad Software, La Jolla, CA). Data were compared across conditions with Student's t tests or chi-square tests. Survival rate of mice were compared by log-rank test. p values <0.05 were regarded as significant. All data were analyzed in SPSS v. 19.0 software (Chicago, IL).