Loratadine inhibits *Staphylococcus aureus* virulence and biofilm formation

**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

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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 μM 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 μM, 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. Overexpression 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, boeravinoine 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 μg/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* 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 μM] or not) was inoculated into 96-well polystyrene microtiter plates with TSBG (trypsin 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 μM loratadine (position: S0101755 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 ≥5 μM (Figure S1B and 1A) but only inhibited growth of *S. aureus* at concentrations ≥50 μM (Figure 1B). In a parallel experiment, 25 μM 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 μM 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 μM) did not disrupt established biofilms of 12 *S. aureus* strains, alone or in combination with antimicrobials (at 8× 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 ≥25 μM (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-α, IL-1β, and IL-10) in the bronchoalveolar lavage fluid of model mice (Figure 3).
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.
Because the difference between desloratadine and loratadine is that the former is missing a side-chain carbamate moiety relative to the latter (Iesce 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.

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

| Biofilm-positive isolates | No. (%) of S. aureus isolates with biofilms inhibited by loratadine (25 μM) |
|---------------------------|------------------------------------------------------------------|
| Weak biofilm producers    | Mild inhibition Moderate inhibition Pronounced inhibition Total inhibition |
| (n = 130)                 | 8 (6.2) 74 (56.9) 47 (36.1) 129 (99.2) |
| Medium biofilm producers  | 0 (0.0) 8 (9.1) 80 (90.9)* 88 (100.0) |
| (n = 88)                  |                                                         |
| Strong biofilm producers  | 0 (0.0) 0 (0.0) 37 (100.0)** 37 (100.0) |
| (n = 37)                  |                                                         |
| Total (n = 255)           | 8 (3.1) 82 (32.2) 164 (64.3) 254 (99.6) |

Note: Inhibition rate (%) of biofilm formation (OD570 of wild type - OD570 of treated isolate)/OD570 of wild type. Mild inhibition, 10% ≤ biofilm formation <40% inhibited; moderate inhibition, 40% ≤ biofilm formation <70% inhibited; pronounced inhibition, biofilm formation ≥70% inhibited.

*Medium versus Weak producers, p < 0.001.

**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 (Iesce 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 OD600 analysis.
(B) Images show spun-down cells.
(C) Pigmentation-reducing effects of loratadine on S. aureus USA300, detected by OD450 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 (± 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.
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 S6). 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 µM 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...
Table S4 (RNA levels of genes between the loratadine treated and the untreated of \textit{S. aureus} USA300 strains). Among these genes, loratadine-treated \textit{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 \textit{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 modulin PSM\textsubscript{b}, and PSM\textsubscript{d}. The RNA levels of the following competence proteins were also reduced significantly by loratadine in most of the 25 clinical isolates: ComK, lipase, thermoneuclease, and four transcriptional regulatory genes \textit{agrA}, \textit{saeR}, \textit{sarA}, and \textit{sigB}.

**Genetic mutations in loratadine-induced isolates of \textit{S. aureus}**

To explore the potential target of loratadine in \textit{S. aureus}, loratadine non-sensitive isolates were induced \textit{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 \textit{S. aureus} SA113) induced under a high concentration (500 \textmu M) of loratadine were selected. Crystal violet staining analyses revealed that the biofilm...
formation ability of two clones (81-1 and 81-6) recovered to levels of the control clone (0-0) and 25 μM loratadine did not inhibit their biofilm formation (Figure 7B). In *S. aureus* USA300 strain clones induced under high-concentration (500 μM) 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 (Keinho¨rster 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
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 µM) on $mgrA$-mt overexpression strain (SA113-pRB475-mgrA-nt) 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
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-α, 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 β-lactam antibiotic efficacy against MRSA and potentiated both β-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

| Gene_ID | Description/function | SA113 RNA-seq | SA113 RT-qPCR | USA300 RNA-seq | USA300 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 (PSMβ) | 0.0160 | 0.4015 | 0.0171 | 0.0115 |
| SACOL_RS10560 | Delta-lysin family phenol-soluble modulin (PSMδ) | 0.0336 | 0.2764 | 0.0549 | 0.0461 |
| SACOL_RS06070 | 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_RS14075 | 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 sib | 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).
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 $\geq 25 \mu M$ (9.572 $\mu g/mL$) 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 formation.

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**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 $\mu M$ and then successively increased to high concentration (500 $\mu M$). SNP, single-nucleotide polymorphism.

(B) The 23 clones from generation 81 of *S. aureus* SA113 strain that were induced under the high concentration (500 $\mu M$) of loratadine were selected, and the inhibitory effect of loratadine (25 $\mu M$) 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.
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 3. Mutations in SA113 strain clones detected by whole-genome sequencing |
|---|---|---|---|---|
| Gene_ID | Locus_tag | SwissProt (CP000253.1) | Nucleotide mutation | Amino acid mutation (type) |
| SA113_GM000528 | SAOUHSC_01,012 | Q2FI10; Phosphoribosylformylglycinamidine 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 | QSHCZ5; 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 µM 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.

| 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.
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-sensitve 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-Stop 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.

**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 OD570 value of Control – OD570 value of the strains treated with loratadine 25 μM/the OD570 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.
STAR METHODS
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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-LO22) 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 |
|---------------------|--------|------------|
| **Experimental models: organisms** | | |
| C57BL/6J mice | Shanghai Jiesijie Laboratory Animal Co., Ltd (Shanghai, China) | N/A |
| **Bacterial strains** | | |
| Staphylococcus aureus | ATCC | ATCC 29213 |
| Staphylococcus aureus | ATCC | ATCC 35556 |
| Staphylococcus aureus | ATCC | ATCC BAA-1556 |
| Staphylococcus aureus | the 6th Affiliated Hospital of Shenzhen University Health Science Center | clinical isolates |
| Escherichia coli | TaKaRa Biotechnology, Dalian, China | DC108 |
| **Chemicals, peptides, and recombinant proteins** | | |
| 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 e5, 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 |
| **Critical commercial assays** | | |
| Mouse Tumor Necrosis Factor α (TNF-α) elisa kit | Andy Gene, Beijing, China | AD3051Mo |
| Mouse Interleukin 1β (IL-1β) 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 |
| **Deposited data** | | |
| Raw RNA-Seq data | This paper | Sequence Read Archive (SRA) database: PRJN587170 |
| Raw whole-genome sequencing data | This paper | Sequence Read Archive (SRA) database: PRJNA649959 |

S. aureus COL reference genome

Genome Reference

https://www.ncbi.nlm.nih.gov/nuccore/CP000046.1?report=graph

(Continued on next page)
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).

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 μL of the S. aureus USA300 suspension (1.5 x 10^10 colony forming units/mL). 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. 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), cefazolin 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 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 μL/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 μL/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 48 h 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 dyn/cm^2 for 3 s. After a 1-h incubation at 37°C to allow bacteria attachment, fresh TSBG medium containing loratadine (25 μM) was set to flow from the inlet well at a shear setting of
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 (OD450). 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 OD600 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 OD550 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 × 10^6 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)log10 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 RNasy minikit (Qiagen, Hilden, Germany).
RNA-seq was conducted according to the Illumina RNA sequencing sample preparation guide. Total RNA samples were treated with RNase-free DNase I (TaKaRa). cDNA libraries were prepared with an mRNA-seq sample preparation kit (Illumina) and 50-cycle sequencing was performed by an Illumina HiSeq 2500 sequencer according to the manufacturer’s protocols. Raw sequencing data were processed in Illumina data collection software. RNA-seq of each sample was performed in three independent experiments.

**RNA-seq data analysis and verification**

Raw sequencing reads were preprocessed by filtering out rRNA reads, sequencing adapters, short fragment reads, and other low-quality reads. The remaining reads were mapped to the *S. aureus* COL (CP000046.1) reference genome (*S. aureus* COL strain with strong biofilm forming ability, and has also been used in virulence study of *S. aureus* (Efthimiou et al., 2019; Ulhuq et al., 2020), thus was chose to analyze RNAseq results of genes related to biofilm formation or virulence of *S. aureus*) on the NCBI website with Bowtie2 software (version 2.0.5) based on a local alignment algorithm. The alignments were further processed with BED Tools to determine transcript expression levels and differential expression between each two of the three samples. Differential expression of all transcripts was quantified in DEGseq (version 2.16.1), and fold-change values are presented. RNA-seq results were validated by reverse transcriptase quantitative polymerase chain reactions (PCRs) with the SYBR Premix Ex Taq II Kit (TaKaRa Biotechnology, Dalian, China) on the Mastercycler ep realplex system (Eppendorf) with the primers listed in Table S6. The validation PCRs were performed in triplicate at least three times.

**In vitro induction of loratadine non-sensitive clones**

*S. aureus* SA113 and USA300 strains were subcultured serially in TSB containing loratadine. The initial inducing concentration of loratadine was 25 μM; the concentration was then increased successively to high concentrations up to 500 μM. Strains in each concentration were cultured for 3–5 passages before being exposed to the next concentration. SA113 and USA300 *S. aureus* were subcultured serially in loratadine-free TSB as control strains. Isolates from the last passage with each concentration of loratadine were picked and cultured successively on tryptic soy agar plates without loratadine for two passages. We assessed biofilm formation of SA113 clones and assessed pigmentation and hemolysis of USA300 clones, and then determined the inhibitory effects of loratadine on these variables. Clones with loratadine-unaffected biofilm formation or pigmentation and hemolysis were kept frozen at −80°C in glycerol containing (35%) TSB.

**Whole-genome sequencing detection of mutations in loratadine non-sensitive clones**

The genomic DNA of two *S. aureus* SA113 clones (A3-81.1 and A3-81.6) was extracted with a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). A total amount of 1μg of DNA per sample was used as input material for DNA sample preparations. We generated sequencing libraries using NEBNext® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer’s recommendations. Whole genomes were sequenced in an Illumina HiSeq2500 sequencer. Coding genes, repetitive sequences, non-coding RNAs, genomics islands, transposons, prophages, and CRISPR (clustered regularly interspaced short palindromic repeat) sequences were predicted by the following software/tools: RepeatMasker (http://www.repeatmasker.org/), Tandem Repeats Finder, tRNAscan-SE, rRNAmer, Rfam database, IslandPath-DIOMB, transposon PSI, PHAST, and CRISPRFinder. Gene functions were predicted by referring to the following databases: GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes), COG (Clusters of Orthologous Groups), NR (Non-Redundant Protein Database databases), TCDB (Transporter Classification Database), and Swiss-Prot. Genomic alignments between each sample genome and a reference genome (*S. aureus* NCTC 8325, GenBank: CP000253.1) were performed with MUMmer and LASTZ tools. Single nucleotide polymorphisms, insertions, deletions, and structural variation annotations were identified based on inter-sample genomic alignment results by MUMmer and LASTZ.

**Expression of MgrA protein in vitro**

Full-length *mgrA* was amplified from its ATG start codon by PCR with the following primers (5’ → 3’): forward, GACTGGTGACAGCACAATGGGGTCGCCGATCCATGTCTGATCAACATAATTTAAAAGAACAGCTATGCTTTAG; reverse, GATCTCAGTGGTGCTGCTGCGAGTCTGATCAACATAATTTAAAAGAACAGCTATGCTTTAG. The resultant PCR amplicons (500 base pairs) were purified and digested with BamHI and Xhol endonucleases,
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-β-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 (1μL, 1mM) was incubated at 37°C for 0.5 h with 100 μL of 0.5 μM 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 bicarbonate. 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× 50 mm, 3.5μm, 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).