Published in final edited form as:

Nat Med. 2012 May ; 18(5): 816–819. doi:10.1038/nm.2692.

MRSA epidemic linked to a quickly spreading colonization and virulence determinant

Min Li^{1,§}, Xin Du^{1,§}, Amer E. Villaruz², Binh An Diep³, Decheng Wang⁴, Yan Song¹, Yueru Tian¹, Jinhui Hu¹, Fangyou Yu⁵, Yuan Lu^{1,*}, and Michael Otto^{2,*}

¹Department of Laboratory Medicine, Huashan Hospital, Shanghai Medical College, Fudan University, 12 Central Urumqi Road, Shanghai 200040, People's Republic of China

²Pathogen Molecular Genetics Section, Laboratory of Human Bacterial Pathogenesis, National Institute of Allergy and Infectious Diseases, U. S. National Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20892, USA

³Division of Infectious Diseases, Department of Medicine, University of California, San Francisco, 1001 Potrero Avenue, Box 0811, San Francisco, CA 94110, USA

⁴Key Laboratory of Medical Molecular Virology, Institutes of Medical Microbiology and Biomedical Sciences, Shanghai Medical College, Fudan University, Shanghai 200032, People's Republic of China

⁵Department of Laboratory Medicine, The First Affiliated Hospital of Wenzhou Medical College, Wenzhou 325000, People's Republic of China

Abstract

The molecular processes underlying epidemic waves of methicillin-resistant *Staphylococcus aureus* (MRSA) are poorly understood¹. While a major role has been attributed to the acquisition of virulence determinants by horizontal gene transfer², there are insufficient epidemiological and functional data supporting that concept. We here report the spread of clones containing a previously extremely rare^{3,4} mobile genetic element-encoded gene, *sasX*. We demonstrate that *sasX* has a key role in MRSA colonization and pathogenesis, significantly enhancing nasal colonization, lung disease, and abscess formation, and promoting mechanisms of immune evasion. Moreover, we observed the recent spread of *sasX* from sequence type (ST) 239 to invasive clones belonging to other STs. Our study identifies *sasX* as a quickly spreading critical determinant of MRSA pathogenic success and a promising target for therapeutic interference. Importantly, our results provide proof-of-principle that horizontal gene transfer of key virulence determinants drives MRSA epidemic waves.

Users may view, print, copy, download and text and data- mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

*corresponding authors: Michael Otto Tel.: (301) 443 5209 Fax: (301) 480 3633 motto@niaid.nih.gov Yuan Lu Tel.:

+86-21-52888323 Fax: +86-21-62498118 yuanlu@hsh.stn.sh.cn.

§equal contribution.

Author contributions

M. L., X. D., A. E. V., D. W., Y. S., Y. T., J. H., and F. Y. conducted experiments. M. L., B. A. D., Y. L., and M. O. planned and supervised experiments. M. O. wrote the paper.

S. aureus remains a dangerous pathogen, causing a multitude of serious and sometimes life-threatening diseases around the globe. This is mostly due to widespread antibiotic resistance, which considerably complicates the treatment of *S. aureus* infections⁵. MRSA strains in particular represent a major problem for public health systems, as they combine resistance to methicillin and other β -lactam antibiotics with relatively minor fitness costs, resulting in high infiltration of hospital and community settings⁶. Remarkably, in the U.S., the estimated number of deaths due to MRSA infections exceeds that due to HIV/AIDS⁷. While molecular typing and genome sequencing have provided better insight into MRSA epidemiology in recent years⁸, we have a severe lack of knowledge regarding the mechanisms by which specific molecular determinants cause the spread and pathogenic success of MRSA clones.

Only a very limited number of MRSA clones, belonging to five clonal complexes, are responsible for the majority of MRSA infections worldwide¹. Specific STs are characteristic for a given geographical location. In China, as in most Asian countries, ST239 is predominant⁹. Recently, genome sequencing of an ST239 strain revealed a novel gene of unknown function (SATW20_21850), which is present in only three of the 43 global ST239 strains that have been sequenced^{3,4,10}, and absent from all other known *S. aureus* genomes³. Based on its amino acid sequence, which contains a signal peptide and an LPXTG surface-anchoring motif, this gene encodes a secreted, surface-anchored protein of 15 kDa in its processed form. It is located at the 3' end region of a 127.2-kb Φ SP β -like prophage, which lacks other virulence-associated genes³. There is no similarity of the surface protein to other proteins found in the NCBI databases except for the highly similar, Φ SP β -encoded SesI of *S. epidermidis* RP62A¹¹. We termed the gene *sasX* and showed that it is absent from other major global MRSA strains by analyzing a series of strains from divergent clonal and geographical backgrounds (**Supplementary Spreadsheet 1** online).

Prompted by the epidemiological connection of *sasX* to MRSA ST239, we here evaluated the hypothesis that *sasX* represents a key factor determining the spread of MRSA in China⁹. To that end, we determined ST distribution and frequency of *sasX*-positive clones among > 800 randomly selected *S. aureus* isolates from patients with *S. aureus* infections at three large teaching hospitals located in eastern China between 2003 and 2011 (**Fig. 1a**). The situation in China in terms of MRSA infection rate and dominance of the ST239/SCC_{mec}-III (Brazil/Hungary) clone is largely characteristic of that in most other Asian countries except Japan and South Korea, and Australia^{9,12-15}. Accordingly, ST239 clones were also predominant among the isolates investigated in our study, with ST5 being the second most frequently isolated ST (**Supplementary Fig. 1** online). Notably, the frequency of *sasX*-positive invasive *S. aureus* clones increased significantly from 2003 to 2011 (from 19% to 31%, $P = 0.0026$). Among MRSA isolates, the frequency of *sasX* increased from 21% to 39% ($P = 0.0028$), while the increase among methicillin-sensitive *S. aureus* (MSSA) was not significant (**Fig. 1b**). Furthermore, *sasX* was almost exclusively found among ST239 strains in 2003-2005, while its frequency among isolates of several other STs considerably increased since then (increase from 5% to 28% of non-ST239 clones among *sasX*-positive *S. aureus* between 2003-2005 and 2009-2011, $P = 0.0048$; **Fig. 1c**). Moreover, presence of *sasX* was extremely rare (only one positive clone among 169 tested) among a collection of community isolates from healthy individuals in the same areas (**Supplementary**

Spreadsheet 1 online), indicating that *sasX*-positive clones spread predominantly within the hospital setting. Finally, we did not detect ST239 clones among community isolates, indicating that this ST is linked to hospital infections. Analysis of genes surrounding *sasX* in randomly selected clones of different STs showed that the horizontal transfer of *sasX* always occurs linked to the Φ SP β -like prophage with a conserved phage insertion site, although occasional deletions within the phage were observed (**Supplementary Table 1** online). Together, these findings demonstrate an epidemic wave of hospital-associated *sasX*-positive MRSA in China. Furthermore, they indicate events of horizontal gene transfer resulting in the spread of *sasX* from ST239 to other STs and a causative link between the presence of *sasX* and invasiveness.

To understand the link between *sasX* and infection, we set out to elucidate the biological role of *sasX*. We first demonstrated that the SasX protein is expressed at the *S. aureus* cell surface using immunofluorescence and immunoblot detection (**Fig. 2a,b**). Surface proteins in *S. aureus* fulfill a variety of functions that include first and foremost adhesion to host tissue¹⁶. In particular, adhesion to nasal epithelial cells is of major importance for the colonization, spread and virulence potential of *S. aureus* strains, inasmuch as the nares are the predominant location of *S. aureus* colonization in the human body¹⁷ and there is an epidemiological link between nasal carriage and infection¹⁸. However, the molecular basis of nasal colonization is incompletely understood and there are only a few molecular factors of *S. aureus* that have been identified as potentially responsible for nasal colonization, such as teichoic acids¹⁹ and specific surface proteins²⁰⁻²². To measure the impact of *sasX* on nasal colonization, we constructed isogenic *sasX* deletion mutants in representative (**Supplementary Fig. 2** online) ST239 and ST5 strains. Adhesion of the *sasX* mutant strains to human nasal epithelial cells in vitro was significantly impaired compared to the wild-type strains, and restored in *sasX*-complemented mutant strains (**Fig. 2c**). Furthermore, recombinant SasX protein blocked adhesion of *S. aureus* to human nasal epithelial cells (**Fig. 2d**; **Supplementary Fig. 3** online). Moreover, *sasX* had a significant impact on in-vivo nasal colonization in separate and competitive colonization models (**Fig. 2e**). These data demonstrate that the SasX surface protein promotes nasal colonization.

In addition to facilitating adhesion to host tissue, *S. aureus* surface proteins may cause intercellular bacterial aggregation, a phenotype with multiple consequences, such as increased biofilm formation and immune evasion capacity²³⁻²⁵. Indeed, we found that *sasX* facilitated the formation of large bacterial aggregates (**Fig. 3a**; **Supplementary Fig. 4** online) and promoted biofilm formation (**Fig. 3b**). Interestingly, *sasX* also facilitated primary attachment to an abiotic surface (**Fig. 3c**), which is in keeping with our finding that *sasX* expression was highest during early growth, and decreased afterwards (**Supplementary Fig. 5** online). Moreover, phagocytosis of the wild-type strain by human neutrophils was significantly lower than that of the *sasX* mutant strain (**Fig. 3d,f**). Accordingly, survival in human blood (**Fig. 3e**) and lysis of human neutrophils as a sign of prolonged bacterial survival after phagocytosis (**Fig. 3g**) were significantly increased in the wild-type strain compared to the *sasX* deletion mutant. Notably, these effects are most likely caused predominantly by the aggregation phenotype and not by a gene regulatory effect, as *sasX* did not impact expression of the *ica* genes encoding biofilm exopolysaccharide

biosynthesis, the biofilm and virulence regulator *agr*²⁶, or a series of cytolysins and other surface protein genes (**Supplementary Fig. 2** online). Together, these data show that *sasX* facilitates intercellular aggregation, leading to a significant enhancement of *S. aureus* immune evasion mechanisms.

S. aureus most frequently causes skin and respiratory infections²⁷. Correspondingly, these were also the most abundant types of infection detected in our study (**Supplementary Fig. 6** online). Among infections caused by *sasX*-positive clones, respiratory and pleural-cavity infections as well as abscesses were most prominent (significant difference by type of infection, $P = 0.0139$, chi square test). Therefore, to determine whether *sasX* is a virulence factor during *S. aureus* infection, we selected to perform murine abscess and lung infection models. Mice infected with the wild-type strains developed abscesses that were significantly larger than those in mice infected with the *sasX* mutant strains (**Fig. 4a**). Furthermore, microscopic evaluation showed increased infiltration of inflammatory cells in the abscesses caused by the wild-type strain (**Fig. 4d**). Of note, the strong impact of *sasX* on abscess size is similar to that observed with potent *S. aureus* toxins such as α -toxin²⁸ or phenol-soluble modulins²⁹. In addition, lung wet weight/body weight ratios, degree of inflammation in the lung as assessed by tumor necrosis factor α concentration, and histological examination showed that *sasX* is also a major factor influencing *S. aureus* pathogenesis in the lungs (**Fig. 4b,c,e**). Thus, our data classify *sasX* as a key determinant of MRSA skin and lung infection.

In conclusion, we identified *sasX* as a critical factor promoting nasal colonization, immune evasion and virulence, and a probable main driving force of the Asian MRSA epidemic. Furthermore, our data provide strong support to the notion that acquisition of specific molecular determinants via horizontal gene transfer represents a major mechanism enhancing the pathogenic potential and epidemiological success of MRSA clones. Moreover, based on our data, it is to be expected that the frequency of *sasX* among Asian and international MRSA clones will increase further. We thus propose drug development or vaccination efforts aimed at *SasX* to prevent MRSA colonization and infection.

Methods

For additional procedures, see Supplementary Methods (**Supplementary Methods** online).

Bacterial isolates

807 *S. aureus* isolates were randomly selected from different inpatients with *S. aureus* infections at three different teaching hospitals in China: 100 each from 2004, 2007, and 2010 from Huashan Hospital, Fudan University, Shanghai; 107 from 2006-2008 and 79 from 2009-2010 from Shandong Provincial Hospital, Shandong University, Jinan; 104 from 2003-2005, 105 from 2006-2008 and 112 from 2009-2011 from the first affiliated hospital of Wenzhou Medical College, Wenzhou. 49 *S. aureus* isolates from nasal swabs among more than 500 healthy volunteers in Shanghai and 120 from nasal swabs among more than 1000 healthy volunteers in Wenzhou were also collected as part of a population-based community prevalence study. Characteristics of all isolates are listed in **Supplementary Spreadsheet 1** online. Bacteria were identified as staphylococci by classic microbiological methods: Gram's stain, catalase and coagulase activity on rabbit plasma. *S. aureus* strains

were further categorized by biochemical characterization using the Api-Staph test (BioMérieux, Lyon, France). All strains and plasmids used in this study are listed in **Supplementary Table 2** online. All oligonucleotides used for cloning or polymerase chain reaction procedures are listed in **Supplementary Table 3** online.

Nasal colonization model

All animal work was approved by the ethics committee of Fudan University. Female ICR mice were used for the nasal colonization model (ten mice per group). All mice were six to eight weeks of age at the time of use and received drinking water containing penicillin at $100 \mu\text{g ml}^{-1}$. *S. aureus* strains were grown to mid-exponential growth phase, washed, and resuspended in sterile phosphate-buffered saline (PBS) at 10^7 colony-forming units (CFU) per μl . Mice were anesthetized with isoflurane. The inoculum, which contained 10^8 CFU in $10 \mu\text{l}$ of PBS or PBS alone, was pipetted slowly into the nares of the anesthetized mice without touching the nose with the pipette tip. Seven days after inoculation, ten mice per strain were euthanized and evaluated for nasal carriage of *S. aureus*. The nasal region was wiped externally with 70% ethanol, and the nasal tissue was homogenized in 0.5 ml of tryptic soy broth (TSB). The total number of *S. aureus* CFU per nose was assessed by plating $100 \mu\text{l}$ diluted nasal suspensions on TSB agar containing penicillin ($100 \mu\text{g ml}^{-1}$).

For the competitive nasal colonization model, 15 mice per group were used. *S. aureus* ST239 (HS663) and its isogenic *sasX* deletion mutant were grown to mid-exponential growth phase, washed, and resuspended at equal CFUs in sterile PBS at a total of 2×10^6 CFU per μl . The following steps were similar to above, except that bacteria isolated from the noses at day seven were plated on penicillin and penicillin/tetracycline plates to distinguish wild-type from mutant bacteria.

Lung infection model

Inoculation was performed as described for the colonization model, but with 10^9 CFU per $20 \mu\text{l}$. *S. aureus* cell suspensions were pipetted into the nares of the anesthetized mice ($n=10$ per group) without touching the nose. At 5 days after inoculation, mice were euthanized. The lungs from selected groups of animals were excised, washed with saline, and one lobe was fixed in 10% formalin (Sigma). Paraffin embedding and hematoxylin & eosin (HE) staining were performed as previously described³⁰. The other lobe was homogenized in 0.5 ml of TSB; homogenized lung tissue and serum samples were used for TNF- α detection.

Skin abscess model

Female outbred, immune-competent, hairless mice were used for the abscess model. All mice were between four and six weeks of age at the time of use. *S. aureus* strains were grown to mid-exponential phase, washed once with sterile PBS, then resuspended in PBS at 1×10^9 CFU per $100 \mu\text{l}$. Mice were anesthetized with isoflurane and inoculated with $100 \mu\text{l}$ PBS containing 10^9 live *S. aureus* or saline alone in the right flank by subcutaneous injection. We examined test animals at 24-h intervals for a total of 10 d with a caliper. We applied length (L) and width (W) values to calculate the area of abscesses with the formula $L \times W$. Abscesses as shown in Fig. 4a developed into scarred lesions during the time of the experiment. The sizes of these lesions showed similar differences as the closed abscesses

between the wild-type and *sasX* mutant groups and remained until the end point of the experiment (**Supplementary Fig. 7** online). However, only the sizes of closed abscesses were measured. All animals were euthanized after completion of the entire procedure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank Frank DeLeo for critically reading the manuscript. This study was supported by a grant from the National Clinical Key Subject to Y.L., the National Natural Science Foundation of China (grants 30900026 and 81171623) and the Shanghai Pujiang Program (grant 09PJ1402300) to M.L., and the Intramural Research Program of the National Institute of Allergy and Infectious Diseases (NIAID), U. S. National Institutes of Health (to M.O.).

References

1. Chambers HF, DeLeo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol.* 2009; 7:629–641. [PubMed: 19680247]
2. Diep BA, Carleton HA, Chang RF, Sensabaugh GF, Perdreau-Remington F. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis.* 2006; 193:1495–1503. [PubMed: 16652276]
3. Holden MT, et al. Genome sequence of a recently emerged, highly transmissible, multi-antibiotic- and antiseptic-resistant variant of methicillin-resistant *Staphylococcus aureus*, sequence type 239 (TW). *J Bacteriol.* 2010; 192:888–892. [PubMed: 19948800]
4. Harris SR, et al. Evolution of MRSA during hospital transmission and intercontinental spread. *Science.* 2010; 327:469–474. [PubMed: 20093474]
5. Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest.* 2003; 111:1265–1273. [PubMed: 12727914]
6. DeLeo FR, Chambers HF. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. *J Clin Invest.* 2009; 119:2464–2474. [PubMed: 19729844]
7. Kleven RM, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *Jama.* 2007; 298:1763–1771. [PubMed: 17940231]
8. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. *Trends Microbiol.* 2001; 9:486–493. [PubMed: 11597450]
9. Liu Y, et al. Molecular evidence for spread of two major methicillin-resistant *Staphylococcus aureus* clones with a unique geographic distribution in Chinese hospitals. *Antimicrob Agents Chemother.* 2009; 53:512–518. [PubMed: 19029328]
10. Li Y, et al. Complete genome sequence of *Staphylococcus aureus* T0131, an ST239-MRSA-SCCmec type III clone isolated in china. *J Bacteriol.* 2011
11. Soderquist B, et al. *Staphylococcus epidermidis* surface protein I (SesI): a marker of the invasive capacity of *S. epidermidis*? *J Med Microbiol.* 2009; 58:1395–1397. [PubMed: 19556370]
12. Aires de Sousa M, et al. Frequent recovery of a single clonal type of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. *J Clin Microbiol.* 2003; 41:159–163. [PubMed: 12517842]
13. Arakere G, et al. Genotyping of methicillin-resistant *Staphylococcus aureus* strains from two hospitals in Bangalore, South India. *J Clin Microbiol.* 2005; 43:3198–3202. [PubMed: 16000435]
14. Ip M, Lyon DJ, Chio F, Enright MC, Cheng AF. Characterization of isolates of methicillin-resistant *Staphylococcus aureus* from Hong Kong by phage typing, pulsed-field gel electrophoresis, and fluorescent amplified-fragment length polymorphism analysis. *J Clin Microbiol.* 2003; 41:4980–4985. [PubMed: 14605127]
15. Ko KS, et al. Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. *J Clin Microbiol.* 2005; 43:421–426. [PubMed: 15635004]

16. Foster TJ, Hook M. Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol. 1998; 6:484–488. [PubMed: 10036727]
17. Wertheim HF, et al. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 2005; 5:751–762. [PubMed: 16310147]
18. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of *Staphylococcus aureus* bacteremia. Study Group. N Engl J Med. 2001; 344:11–16. [PubMed: 11136954]
19. Weidenmaier C, et al. Role of teichoic acids in *Staphylococcus aureus* nasal colonization, a major risk factor in nosocomial infections. Nat Med. 2004; 10:243–245. [PubMed: 14758355]
20. O'Brien LM, Walsh EJ, Massey RC, Peacock SJ, Foster TJ. *Staphylococcus aureus* clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. Cell Microbiol. 2002; 4:759–770. [PubMed: 12427098]
21. Wertheim HF, et al. Key role for clumping factor B in *Staphylococcus aureus* nasal colonization of humans. PLoS Med. 2008; 5:e17. [PubMed: 18198942]
22. Corrigan RM, Miajlovic H, Foster TJ. Surface proteins that promote adherence of *Staphylococcus aureus* to human desquamated nasal epithelial cells. BMC Microbiol. 2009; 9:22. [PubMed: 19183486]
23. Foster TJ. Immune evasion by staphylococci. Nat Rev Microbiol. 2005; 3:948–958. [PubMed: 16322743]
24. Otto M. Staphylococcal biofilms. Curr Top Microbiol Immunol. 2008; 322:207–228. [PubMed: 18453278]
25. Otto M. Bacterial evasion of antimicrobial peptides by biofilm formation. Curr Top Microbiol Immunol. 2006; 306:251–258. [PubMed: 16909925]
26. Vuong C, Saenz HL, Gotz F, Otto M. Impact of the *agr* quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis. 2000; 182:1688–1693. [PubMed: 11069241]
27. Moran GJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. N Engl J Med. 2006; 355:666–674. [PubMed: 16914702]
28. Kennedy AD, et al. Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis. 2010; 202:1050–1058. [PubMed: 20726702]
29. Wang R, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med. 2007; 13:1510–1514. [PubMed: 17994102]
30. Koh SS, et al. Molecular classification of melanomas and nevi using gene expression microarray signatures and formalin-fixed and paraffin-embedded tissue. Mod Pathol. 2009; 22:538–546. [PubMed: 19270649]

Figure 1.

Spread of *sasX*-positive clones in China. (a) A total of 807 isolates from three teaching hospitals in eastern China were analyzed for ST, methicillin resistance, and presence of *sasX*. (b) Percentage of *sasX*-positive among MSSA and MRSA isolates. Statistical analysis is by chi square test of all MRSA isolates (average curve, shown in black). The P value including all (MRSA and MSSA) isolates is 0.0026. (c) Percent of *sasX*-positive isolates in STs other than ST239. Statistical analysis is by Fisher's exact test of all isolates (average curve, shown in black).

Figure 2.

The SasX surface protein facilitates nasal colonization. **(a)** Detection of SasX using anti-SasX polyclonal antiserum in an indirect immunofluorescence assay. **(b)** Immunoblot detection of SasX in different subcellular fractions. Black arrows point to the putative SasX protein. Note that surface proteins in this assay run higher than their predicted molecular weights in the processed forms, owing to the linkage to remaining peptidoglycan parts. W, whole cell preparation; S, surface protein fraction; C, cytoplasmic fraction; E, extracellular fraction. **(c)** Adherence of wild-type, isogenic *sasX* mutant, *sasX*-complemented, and control strains to human nasal epithelial cells in vitro. **(d)** Blocking of adherence to nasal epithelial cells by recombinant GST-SasX fusion protein. Purified GST protein alone and denatured GST-SasX fusion protein were used as controls. **(e)** Nasal colonization model. Female ICR mice (n=10 per group in the separate model; n=15 per group in the competition model) were inoculated intranasally with 10^7 CFU of wild-type or isogenic *sasX* mutant strains and bacteria were counted in mice that were euthanized at seven days post inoculation. ST239 HS770 was used for the separate and ST239 HS663 for the competition model. Equal amounts of wild-type and deletion mutant bacteria were mixed for the competition model inoculum. Statistical analyses in (c-e) are by unpaired t-tests.

Figure 3.

SasX promotes bacterial aggregation and mechanisms of immune evasion. (a) Microscopic pictures of 16-h cultures of ST239 wild-type, isogenic *sasX* mutant, *sasX*-complemented, and control strains. (b) Biofilm formation. Cultures were grown for 24 h in microtiter plates. Afterwards, biofilms were measured using crystal violet staining. (c) Primary attachment. The experiment was performed as that shown in (b), but with only 1 h growth. (d) Phagocytosis assay. Phagocytosis by human neutrophils of ST239 or the isogenic *sasX* mutant strain was examined using microscopy (see panel f). (e) Survival in human blood at t=90 min. (f) Microscopical examination of phagocytosis. Slides were stained using a modified Wright-Giemsa stain. At t=90 min, lysed neutrophils were occasionally detected in the ST239 wild-type samples. The 90 min ST239 image shows such a lysed neutrophil with adherent *S. aureus* bacteria. (g) Neutrophil killing. Bacteria from mid-logarithmic growth phase were washed and incubated with human neutrophils at a 100:1 ratio. Neutrophil lysis was determined by measuring release of lactate dehydrogenase (LDH). Statistical analyses in (b-e, g) are by unpaired t-tests.

Figure 4.

SasX is a key virulence determinant during MRSA skin and lung infection. (a,d) Skin infection. Outbred, immune-competent, hairless mice were inoculated with 10^9 CFU of the ST239 or ST5 wild-type and isogenic *sasX* mutant strains. (b,c,e) Lung infection. Female ICR mice were inoculated in the noses with 10^9 CFU/20 μ l of the ST239 or ST5 wild-type and isogenic *sasX* mutant strains. Mice were euthanized at day five post infection for subsequent analyses. (a) Skin infection model, developing abscess areas (see representative images on the right). Differences between corresponding wild-type and mutant abscess sizes were statistically significant at every time point (unpaired t-tests, $P < 0.05$). (b) Lung infection model, lung wet weight/body weight ratios. (c) Lung infection model, concentration of tumor necrosis factor α (TNF- α) in lung tissue samples. (d) Skin infection model, hematoxylin/eosin (HE) stained tissue samples. (e) Lung infection model, HE stained tissue samples. Note increased number of infiltrating inflammatory cells in wild-type sample pictures in (d) and extensive inflammation accompanied by hemorrhagic infiltration and disruption of pulmonary architecture in wild-type sample pictures in (e). Statistical analyses in (b,c) are by unpaired t-tests.